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Genetic and Systematic Study of Viviparus Georgianus (Lea), a Freshwater Snail Species Complex.

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GENETIC AND SYSTEMATIC STUDY OF *VIVIPARUS GEORGIANUS* (LEA), A FRESHWATER SNAIL SPECIES COMPLEX

A Dissertation

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ABSTRACT

Genetic and morphological variation were studied in a brooding (ovoviviparous) and morphologically variable freshwater snail [Viviparus georgianus (Lea)] in the southeastern United States. Eleven populations were clustered into three genetically isolated, allopatric species characterized by 7 to 15 diagnostic loci out of the 38 loci examined. These allopatric species were an eastern species (in eastern and southern Florida), a western species (in the Florida panhandle), and a central species in the Ochlockonee River. Nei's standard genetic distances between species were large (0.23-0.52) compared to within-species distances (0.00-0.06). Moreover, genetic distances between the Ochlockonee River species and other species were larger than the distance between the eastern and western species. Hierarchical F-statistics for differentiation among sites within drainage systems (F_{SD}) of the western and eastern species were large (0.519 and 0.387, respectively). The F_{DT} values (differentiation among drainage systems within the total area sampled) were negative, so most of the intraspecific genetic differentiation was due to differences among populations within drainage systems, rather than to differences among systems. Canonical discriminant analysis of nine shell measurements separated all three species with little overlap. The type specimens of the Viviparus georgianus complex and type locality specimens were compared to the discriminant function and canonical discriminant analyses of shell characters of the studied samples to assign correct species names. The

western species and Ochlockonee River species appear to be Viviparus goodrichi Archer and Viviparus limi Pilsbry, respectively, which were originally described as subspecies of Viviparus contectoides (= Viviparus georgianus). The eastern species is Viviparus georgianus (Lea). The three species can be distinguished by the following morphological characteristics: V. goodrichi has a more globose shell with a larger aperture than V. limi; V. georgianus has shorter aperture height than the other species. Also, these three species can be identified reliably using allozyme characters.

CHAPTER 1

INTRODUCTION

This dissertation describes my research on genetics and systematics of Viviparus georgianus (Lea, 1837), a freshwater snail species complex. The dissertation has two chapters. In Chapter 2, I discuss genetic and morphological variation within and between three allozymically defined species of Viviparus; in Chapter 3, I describe the systematics of the three Viviparus species.

During the course of this research, I tried to accomplish three goals. My first goal was to find evidence of genetic subdivision in the very polymorphic species Viviparus georgianus. Previous research indicates that freshwater gastropods are highly differentiated genetically. These genetic discontinuities may or may not be related to speciation events. Despite decades of intense research on genetic subdivision, speciation mechanisms and their phenotypic consequences are still poorly understood. My second goal was to correlate the observed genetic subdivision with morphological differences. A degree of concordance between genetic and morphological differences not only characterizes Viviparus speciation with or without morphological differentiation but also evaluates the systematic value of morphological characters in Viviparus. My last goal was to assign the three Viviparus species to their proper taxa.

In Chapter 2, the three species identified by electrophoretic data were called Viviparus georgianus (Lea),

Viviparus limi Pilsbry, and Viviparus goodrichi Archer according to the analysis in Chapter 3. In Chapter 3, however, they were referred to as the eastern, Ochlockonee River, and western species, respectively, based on their distributions only, to distinguish between my own collections and museum specimens.

CHAPTER 2

DIFFERENTIATION OF POPULATIONS

Introduction

Genetic subdivision

Genetic subdivision is of great interest for evolutionary biologists because it is thought to be a fundamental requirement not only for speciation but also for local adaptation. For these reasons it has been studied thoroughly using molecular techniques since Harris (1966) and Lewontin and Hubby (1966) observed high levels of genetic variation in man and Drosophila pseudoobscura, respectively. The amount of genetic divergence among populations is determined by combinations of gene flow, selection, and genetic drift. These three factors have different effects on population differentiation. Gene flow among populations decreases genetic differentiation caused by selection or genetic drift and disrupts locally adapted gene combinations. In contrast, small effective population sizes accelerate allele frequency changes due to genetic drift (Wright, 1931, 1932).

Mollusks are frequently used to study genetic differentiation because they have different levels of gene flow, due to various modes of reproduction, dispersal abilities, and different degree of geographical subdivision. Some marine species have free swimming larvae and exhibit little allozymic divergence over large geographical ranges (Janson, 1987; Liu et al., 1991). The larvae of other species develop in the mantle cavity or pallial oviduct to a crawl-away juvenile stage. Other

species produce benthic egg masses which are anchored to algae or rocks and do not have free swimming larval stages. The latter two groups have limited gene flow, and populations tend to be differentiated (Janson, 1987). Geographic subdivision may occur in any habitat. For example, although marine species inhabit continuous environments, they may be separated by distance, by land, or by differences in hydrostatic pressure, water temperature, or salinity. Terrestrial and freshwater species may be physically separated by water (such as rivers and oceans or any uninhabitable areas) or land barriers, respectively.

A freshwater snail, such as Viviparus georgianus in the southeastern United States, is expected to have a high level of genetic subdivision because of ovoviviparity, low motility, and the geographical separation of different river systems. Previous research indicates that freshwater gastropods are highly differentiated not only among different drainage systems but also within the same drainage system (Chambers, 1980; Dillon and Davis, 1980; Dillon, 1984). V. georgianus is highly polymorphic in shell morphology (Clench and Turner, 1956; Clench, 1962; Clench and Fuller, 1965; Thompson, 1984). This morphological polymorphism may be discontinuous among genetically subdivided populations (if they exist). Shell characters are often unreliable for specific identification because of convergence (Davis, 1982) and environmental effects (Vermeij, 1980). In this study, both allozyme electrophoresis and shell morphometrics were used to compare the degree of differentiation in highly variable freshwater snail populations

separated by geographical barriers, to test the hypothesis that river systems are isolated enough to produce genetic and morphological differentiation, and to test whether there is concordance of genetic and morphological differentiation.

Biogeography

Another aim of this study is to assess whether the genetic subdivision of Viviparus georgianus agrees with patterns inferred from historical biogeography. Such data allow us to decide how important historical biogeography is as a determinant of present genetic structure. Historical biogeography concerns the relationships among geological history and the past and present distributions of organisms. Present geographical distribution has been explained by dispersal (Darlington, 1957, 1965) or vicariance (subdivision by a barrier) models (Croizat et al., 1974; Platnick and Nelson, 1978). In the dispersal model, a barrier is pre-existing, and an ancestral species accidentally crosses the barrier. Isolated populations subsequently differentiate into two allopatric species. In the vicariance model, subdivision occurs within the distribution of an ancestral species. Subdivided populations eventually evolve into two species, as in the dispersal model. These two hypotheses are similar in that each one predicts that speciation should be allopatric. However, the hypotheses make different predictions about how often similar distributional patterns will be found among independently evolving lineages. In the dispersal model, concordant geographic distributions will be found only when two or more species cross the same barrier by

chance. On the other hand, all species existing in subdivided regions may show the coincident distributions in the vicariance model if they had similar distributions before the vicariance event. If the barriers to gene flow reflect a shared geologic history, different taxa should show similar genetic divergence even though they have evolved independently (Avise et al., 1987; Avise and Ball, 1990). These hypotheses should be tested by repeated studies in the same region using various organisms. Of course, present-day (e.g., human mediated) dispersal may obscure historical subdivision.

Repeated findings of similar geographic patterns of genetic polymorphism among a wide range of organisms indicate the influence of shared geologic history (Bermingham and Avise, 1986) and support vicariance models for evolution in the region. On the other hand, differences among species also reflect ecological differences (e.g., dispersal ability), different responses to the same geological events, and differences in speciation events. Although different distributions among monophyletic lineages may indicate dispersal events, dispersal after vicariance events may cause similar patterns of differentiation, so the results must be interpreted cautiously. Differences in dispersal ability directly affect the genetic structure of organisms in the past and present. Additional studies in the same region using organisms which are distantly related and which have different dispersal potential should give a better understanding of the relationship of geological history to organismal evolution. In this study, the genetic subdivision of the freshwater snail Viviparus georgianus was used to test

the hypothesis that invertebrates will have an east-west genetic discontinuity in the southeastern United States, a pattern which has been repeatedly reported in vertebrates (see a review by Avise et al., 1987).

Avise and his colleagues have extensively studied the molecular zoogeography of vertebrates in the southeastern United States and found intraspecific east-west genetic breaks using mtDNA and allozymes (Avise et al., 1987). MtDNA clones were distantly related between the eastern and western regions. Allozyme alleles were also completely different or nearly so between the regions. They studied freshwater fishes (Avise and Smith, 1974; Avise et al., 1984; Bermingham and Avise, 1986), amphibians (Avise et al., 1987), and mammals (Avise et al., 1979; Avise et al., 1983). The freshwater fishes Lepomis punctatus and Lepomis microlophus were differentiated between the Apalachicola and Suwannee rivers by mtDNA genotypes (Bermingham and Avise, 1986). Two other freshwater fish species showed a more westerly mtDNA genetic discontinuity: Amia calva was separated between the Escambia and Apalachicola rivers and Lepomis gulosus was separated between the Alabama/Tombigbee and Escambia rivers (Bermingham and Avise, 1986). Both mtDNA and allozyme data for Lepomis macrochirus showed a genetic discontinuity between peninsular Florida and Alabama (Avise and Smith, 1974; Avise et al., 1984), and a similar discontinuity in the southeastern pocket gopher (Geomys pinetis) was found at the Apalachicola River (Avise et al., 1979). The mud puppy (Necturus alabamensis), and related forms and species showed a mtDNA genetic break from North Carolina to Louisiana (Avise et

al., 1987). Two terrestrial species studied by Avise and his colleagues did not show an east-west genetic discontinuity in mtDNA structure in the southeastern United States: the old field mouse Permyscus polionotus (Avise et al., 1983) and the southern toad Bufo terrestris (Avise et al., 1987).

Swift et al. (1985) studied the distributional limits of 241 freshwater fish species inhabiting 31 river drainage systems in the southeastern United States. The major break in the species-limits occurred between the Apalachicola and Ochlockonee rivers. Clustering analysis based on presence/absence of species revealed two major groups: an eastern group from the Apalachicola to the Ochlockonee and a western group from the Apalachicola to Lake Pontchartrain (see Figure 7.6 in Swift et al., 1985).

Fewer genetic studies of invertebrates have been done in the southeastern United States. Two of three studies of freshwater viviparid mollusks showed east-west genetic breaks. Karlin et al. (1980) studied Campeloma geniculum from the Apalachicola and Ochlockonee River systems and found one fixed and one nearly fixed difference between the river systems out of 20 loci studied. A major genetic separation of Elimia (=Goniobasis) floridensis populations (characterized by fixed allele differences) existed between the Apalachicola and Suwannee river systems, and some fixed allele differences were found within the drainage system also (Chambers, 1980). On the other hand, the unionacean bivalve Elliptio icterina showed little differentiation based on low Nei's (1972) genetic distance between populations from the Escambia to Suwannee

rivers (Davis et al., 1981). However unionacean glochidium larvae are parasitic on fish; therefore extended fish mediated dispersal is expected.

The distributional survey of freshwater mollusks from the Escambia to the Suwannee rivers by Clench and Turner (1956) did not show an apparent east-west discontinuity. Instead, a high amount of endemism was found in the Apalachicola, Choctawhachee, and Escambia river systems, rivers which arise in the uplands. On the contrary, lack of endemism was found in the smaller river systems such as the Ochlockonee and the Suwannee, rivers whose tributaries do not extend far into the uplands. Clench and Turner (1956) concluded that during the Pleistocene interglacial periods, valleys of the smaller rivers were inundated and endemic species survived only in the larger river systems which had stable upper regions.

Besides the intra and interspecific east-west genetic breaks, biogeography in the southeastern United States is highlighted by endemism in peninsular Florida. Neill (1957) and Remington (1968) reviewed endemism in peninsular Florida and listed a large number of animal and plant species and subspecies with restricted ranges. Of course, many other species are non-endemic, being distributed widely in the southeastern United States. In an extensive southeastern distributional study, Clench and Turner (1956) listed many molluscan species which are present in the Apalachicola and/or the Suwannee systems but are absent in central Florida, and five endemic species or subspecies [Villosa villosa amygdala, Viviparus georgianus wareanus, Anodonta cowperiana, Campeloma floridense, and

Goniobasis (=Elimia) vanhyningiana] in central Florida. However, V. georgianus wareanus was synonymized as V. georgianus by Clench and Fuller (1965).

Life history and systematics of Viviparus

The life history of Viviparus georgianus has been studied extensively (Van Cleave and Lederer, 1932; Browne, 1978; Vail, 1978; Jokinen et al., 1982; Pace and Szuch, 1985). Viviparus is dioecious like most prosobranch snails. The life span of female V. georgianus is two to three years and that of the males is one to two years in the northeastern United States. Females are typically larger than males, but V. georgianus in Florida do not show sexual size dimorphism (Vail, 1978). Intraspecific life history differences in clutch size, growth rates, and life cycles were found in viviparid snails in Louisiana (Brown et al., 1989; Brown and Richardson, 1992). All viviparid snails lack planktonic larval stages and are either ovoviviparous or viviparous (Aldridge, 1983). Dispersal potential of viviparid snails seems to be low because of brooding and low motility. Therefore, substantial differentiation among conspecific populations, especially in different drainage systems, is expected.

The two major groups of freshwater gastropods (Phylum Mollusca) are the Prosobranchia and the Pulmonata. Family Viviparidae belong to the Prosobranchia. Viviparids are worldwide in distribution, and five genera (Tulotoma, Viviparus, Cipangopaludina, Campeloma, and Lioplax) are present in North America (Burch, 1989). Only three Viviparus species, Viviparus

georgianus (Lea), Viviparus intertextus (Say), and Viviparus subpurpureus (Say), are native to North America. In Florida and Georgia and from Louisiana and northern Alabama north to the Great Lakes region and the St. Lawrence River (Clench, 1962), Viviparus georgianus are common in small rivers, springs, lakes, and ponds but not in large rivers (Clench and Turner, 1956). V. intertextus is distributed from eastern Texas and throughout the Mississippi River east to the Alabama River, with disjunct populations in the Altamaha River system in Georgia and in other river systems in South Carolina (Clench and Fuller, 1965). The spotty distributions of V. intertextus and V. georgianus do not have much overlap, especially in the southeastern United States (Clench, 1962; Clench and Fuller, 1965). V. subpurpureus is not present in Florida and is distributed mainly in the Mississippi River system (Clench and Fuller, 1965). Two Japanese species, Viviparus malleatus (Reeve) and Viviparus japonicus (v. Martens), were introduced into California prior to 1900 and have spread to the north central and northeastern United States, but not to the southeastern United States (Clench, 1962).

The taxonomic status of Viviparus georgianus has been questioned because of its variable shell morphology (Clench and Turner, 1956; Clench, 1962; Clench and Fuller, 1965; Thompson, 1984). Vail (1977) separated three genera of viviparids (Viviparus, Campeloma, and Bellamya) based on anatomical characters. However, one is hardly able to distinguish species of any of the viviparid genera based on the anatomical literature (Dr. Kenneth J. Boss, Museum of Comparative Zoology, personal communication). Populations of V. georgianus are

highly variable in morphology, although each population tends to be uniform in its characteristics. As a consequence, fourteen (sub)species names have been proposed for various forms based upon color and shell globoseness (see Appendix). Later, the fourteen proposed (sub)species names were synonymized by Clench and Fuller (1965) in their revision of the genus; however, they did not give specific reasons. Now, Viviparus georgianus is recognized as a very polymorphic species, but its validity is still questionable (Thompson, 1984).

Viviparus georgianus was originally described by Lea (1837) as Paludina georgiana based on the type specimen from Hopeton, near Darien, Georgia in the Altamaha River system. Lea (1837) stated, "Shell ventricoso-conical [*sic*], thin, dark horn coloured, smooth; sutures very much impressed; whorls about five; convex; aperture nearly round, white" in his original description. He did not mention bands because the type specimen did not have bands on the shell. His description of the species was modified by Clench and Fuller (1965) to cover all shell variation of V. georgianus presently recognized.

Binney (1865) described Vivipara (=Viviparus) contectoides from Florida (Clench and Fuller, 1965) as "Shell umbilicated, elongately-ovate, rather thin, smooth, shining, the surface scarcely broken by the extremely delicate lines of growth; greenish horn-color, sometimes darker, varied with several longitudinal dark streaks marking the former peristome, and with four well marked brown bands revolving upon the body whirl, of which two only are visible on the penultimate and antepenultimate; under the epidermis of a pale yellowish color,

still plainly showing the bands; spire scalariformly turbinated, apex entire, well defined, obtuse; whirls 5, bulging, regularly and rapidly increasing in length, the last ventricose, more than one-half the shell's length, umbilicated; aperture sub-circular, oblique, about half as long as the body whirl, within white, showing plainly the four revolving bands, the lower one very near its base, none of them reaching the edge of the aperture; peristome dark, thin, acute, made continuous by the dark, thin, exserted callus which connects the terminations, somewhat reflected at the umbilicus." Binney (1865) distinguished V. contectoides from Viviparus georgianus by the perfect apex, the greater globoseness of whorls, and the more shining surface of V. contectoides.

Pilsbry (1916) briefly described Viviparus contectoides compactus as a new subspecies from Dougherty [Co.?], Georgia. This is the first description from the Apalachicola River system. His description was that the shell was compactly coiled, imperforate; whorls a little less inflated than in Viviparus contectoides. Later, the subspecies name was corrected as Viviparus contectoides limi because of homonyms (Pilsbry, 1918).

Another subspecies (Viviparus contectoides goodrichi) in the Apalachicola River system was described from the Chipola River, Florida based on shell morphology (Archer, 1933). V. contectoides goodrichi is distinguished from V. contectoides by having larger size, more globoseness, whorls broadly shouldered instead of rounded and convex, and by being not as umbilicate as V. contectoides (Archer, 1933). Moreover, V. contectoides

goodrichi is characterized by a dark brown shell color and three dark brown bands visible in some individuals (Archer, 1933). Clench and Turner (1956) noted that they found individuals intermediate between Viviparus contectoides limi and V. contectoides goodrichi and between these taxa and Viviparus georgianus.

Clench and Fuller (1965) synonymized fourteen species and subspecies of the Viviparus georgianus complex (see Appendix) including V. contectoides, V. contectoides limi, and V. contectoides goodrichi. In the V. georgianus description, they stated, "Shell subglobose in outline and varying in size, large specimens reaching about 44 mm. ... in length, imperforate or with a narrow, slit-like umbilicus. Usually rather thin in structure, but strong and smooth. Color yellowish or olivaceous green to dark brownish green, banded or uniform in color. Banded specimens usually have four dark, reddish brown bands, fairly evenly spaced. Whorls 4 to 5, strongly convex and generally with a slight shoulder. Spire somewhat extended and produced at an angle of from 50 to 65. Aperture ovate to subcircular. Outer lip thin, parietal lip consisting of a thickened glaze. Columella narrow and arched. Suture deeply indented. Sculpture consisting only of fine growth lines. Young specimens with a few spiral threads which eventually disappear as they grow older. Operculum corneous, thin, with concentric growth lines and submarginal nucleus."

The previous systematic studies of the Viviparus georgianus complex were solely based on shell morphology. In this chapter, genetic data of V. georgianus were used to examine validity of

the revision of V. georgianus by Clench and Fuller (1965). Moreover, the genetic data were utilized to understand the pattern of morphological variation of V. georgianus.

In this study, I used allozyme electrophoresis to study the population genetic structure of 11 collections of the Viviparus georgianus complex. All electrophoresed snails were also used for comparison of shell morphology. Three allopatric species were recognized in the Viviparus georgianus complex based on the allozyme and shell morphology data. The eastern, Ochlockonee River, and western species were identified as Viviparus georgianus, Viviparus limi, and Viviparus goodrichi in the later chapter. Therefore, I use the same nomenclature in this chapter. V. limi and V. goodrichi were originally described as subspecies of V. contectoides (=georgianus).

Materials and Methods

Eleven collections comprising 355 snails were made in August 1989 and June 1990 from six drainage systems in Florida and Georgia (Figs. 2.1 and 2.2, and Table 2.1). Snails were transported alive to Louisiana State University in Baton Rouge, LA and frozen at -70°C until electrophoresed. Snails (N = 30 per site when available) were weighed and dissected for electrophoresis. Preliminary experiments suggested that banding patterns of adenylate kinase and arginine kinase were suboptimal in whole body extracts, so digestive gland and foot tissue were removed separately from each snail to make enzyme extracts. Sex was determined by presence of modified right tentacles in males

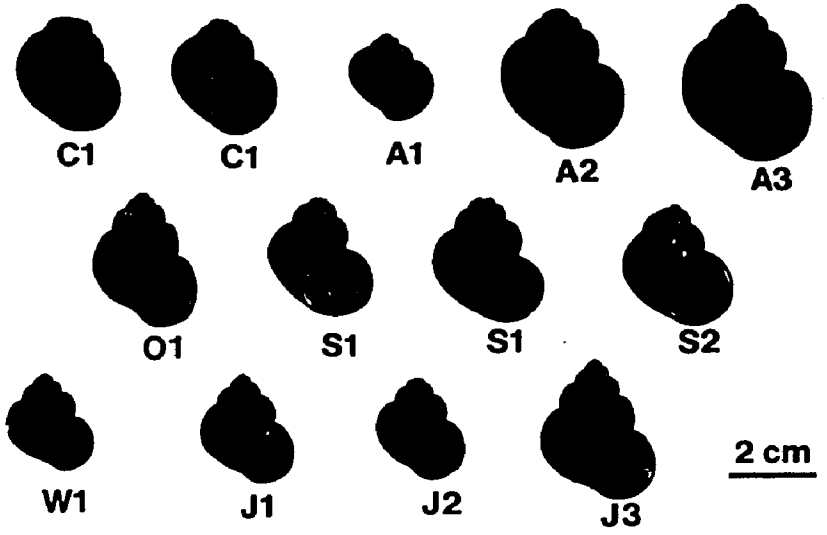


Fig. 2.1. Shell morphology variation of the three Viviparus species in the southeastern United States. V. goodrichi: Homes River (C1), Chipola River in Jackson Co. (A1), Chipola River in Calhoun Co. (A2), and Spring Creek (A3); V. limi: Lake Talquin (O1); V. georgianus: Santa Fe River (S1), Withlacoochee River of Suwannee (S2), Withlacoochee River in southern Florida (W1), Silver River (J1), Wekiva River (J2), and Lake Monroe (J3).

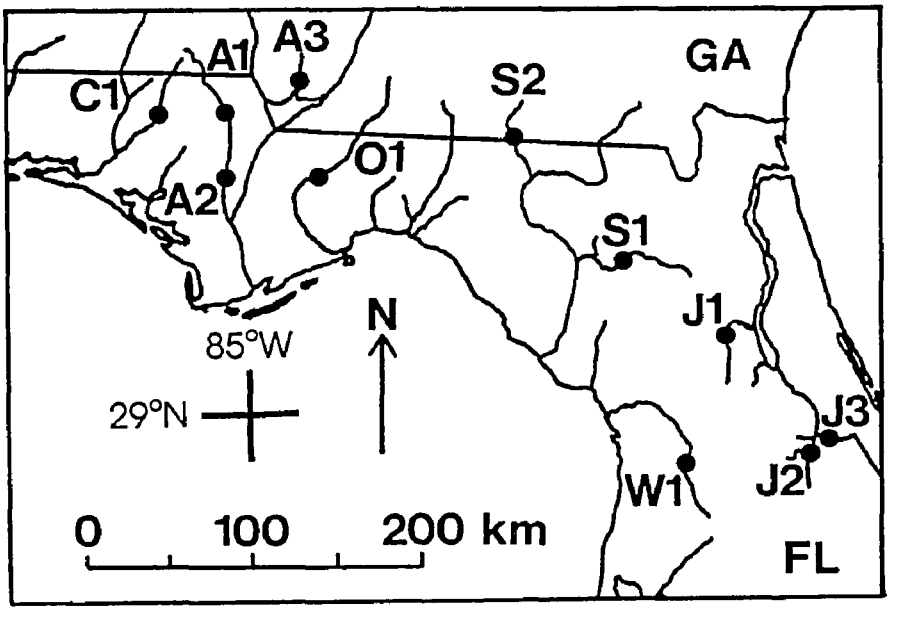


Fig. 2.2. Location of 11 sites in the southeastern United States. Viviparus goodrichi: Homes River (C1), Chipola River in Jackson Co. (A1), Chipola River in Calhoun Co. (A2), and Spring Creek (A3); V. limi: Lake Talquin (O1); V. georgianus: Santa Fe River (S1), Withlacoochee River of Suwannee (S2), Withlacoochee River in southern Florida (W1), Silver River (J1), Wekiva River (J2), and Lake Monroe (J3).

Table 2.1. Three Viviparus species from Florida and Georgia examined for electrophoretic and morphological analyses.

Code and locality	Collection date (mo./yr.)	No. snails examined	Voucher number[a]
Viviparus goodrichi			
Choctawhachee River drainage			
C1 Homes River at US Highway 90 (30°47'N, 85°37'W) Washington Co., FL	8/89	30	UF 193505
Apalachicola River drainage			
A1 Chipola River near Marianna (30°49'N, 85°14'W) Jackson Co., FL	8/89	30	UF 193506
A2 Chipola River at FL Highway 20 (30°26'N, 85°10'W) Calhoun Co., FL	6/90	30	UF 193507
A3 Spring Creek at GA Highway 91 (31°10'N, 84°45'W) Miller Co., GA	8/89	30	UF 193508
Viviparus limi			
Ochlockonee River drainage			
O1 Lake Talquin at Coe Landing (30°25'N, 84°34'W) Leon Co., FL	8/89 & 6/90	7 & 23	UF 193509 & UF 193510
	8/89 & 6/90	9[b] & 21[b]	UF 193511 & UF 193512

Table 2.1. (continued)

Code and locality	Collection date (mo./yr.)	No. snails examined	Voucher number
Viviparus georgianus			
Suwannee River drainage			
S1 Santa Fe River at US Highway 27 & US Highway 441 (29°51'N, 83°38'W) & (29°51'N, 83°36'W) Columbia Co., FL	8/89	14 & 16	UF 193513 & UF 193514
S2 Withlacoochee River at GA Highway 31 (30°38'N, 83°18'W) Lowndes Co., GA	6/90	30	UF 193515
Withlacoochee River drainage			
W1 Withlacoochee River[c] at Co. Highway 48 (28°43'N, 82°14'W) Sumter Co., FL	8/89 & 6/90	3 & 26	UF 193516 & UF 193517
St. Johns River drainage			
J1 Silver River at FL Highway 40 (29°13'N, 82°03'W) Marion Co., FL	8/89	30	UF 193518
J2 Wekiva River at FL 46 (28°49'N, 81°25'W) Seminole Co., FL	6/90	26	UF 193519
J3 Lake Monroe at US Highway 92 & 17 (28°50'N, 81°19'W) Volusia Co., FL	6/90	30	UF 193520

[a]Florida State Museum, University of Florida, Gainesville.
[b]For the morphological analysis only.
[c]This is not the same river as the Withlacoochee River of Suwannee.

or by presence of brooded young in females during dissection. Shells were saved individually for later morphological analysis. Procedures for tissue-extract preparation, and horizontal starch gel electrophoresis were similar to those of Selander et al. (1971), Harris and Hopkinson (1976), and Murphy et al. (1990) with minor modifications. Thirty-eight enzyme loci were resolved in this study (Table 2.2). Multiple loci encoding the same enzyme (isozymes) were designated by consecutive numbers, with "1" denoting the fastest migrating isozyme. Alleles within each locus were scored by designating the most common allele in the Silver River collection (J1) as 100, all other alleles being numbered according to their relative anodal distance from the reference allele. Genetic nomenclature is according to Shaklee et al. (1990). All enzyme systems exhibited anodal migration except *AAT-2, IDH-2, LDH-2,* and *MDH-2.*

The genetic differentiation of populations was compared using diagnostic loci, Nei's (1978) unbiased and modified Rogers' (Wright, 1978) genetic distances, and principal component analysis (SAS Institute Inc., 1990). Diagnostic loci are those for which individuals of a given genotype can be assigned to a population or species with at least a 99% probability of correct assignment (Ayala and Powell, 1972). In this study, the genotypic frequencies of all individuals in each species were used to identify diagnostic loci. Fixed allele differences at diagnostic loci are the most definitive evidence against gene flow between populations or species. Nei's genetic distance is a measure of codon substitution per locus if the rate of gene substitution per locus is the same for all loci

Table 2.2. Enzymes assayed by electrophoresis in the Viviparus georgianus complex.

Enzyme abbreviation[a]	Enzyme	E.C. no.[b]	Number of loci	Tissue[c]	Buffer[d]
AAT	Aspartate aminotransferase	2.6.1.1	2	D	TC
AH	Aconitase hydratase	4.2.1.3	1	D	TBE
AK	Adenylate kinase	2.7.4.3	1	M	TC
ALAT	Alanine aminotransferase	2.6.1.2	1	D	TME
ALP	Alkaline phospatase	3.1.3.1	1	D	TBE
AO	Aldehyde oxidase	1.2.3.1	1	D	TBE
ARK	Arginine kinase	2.7.3.3	1	M	TC
ESTD	Esterase-D	3.1.1.-	1	D	TME
FH	Fumuarate hydratase	4.2.1.2	1	M	TBE
βGAL	β-Galactosidase	3.2.1.23	1	D	AC
βGALA	β-N-Acetylgalactosaminidase	3.2.1.53	1	D	TC
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	1	M	TC
βGLUR	β-Glucuronidase	3.2.1.31	1	D	AC
αGLUS	α-Glucosidase	3.2.1.20	1	D	TME
G3PDH	Glycerol-3-phosphate dehydrogenase	1.1.1.8	1	D	TBE
G6PDH	Glucose-6-phosphate dehydrogenase	1.1.1.49	1	M	TBE
GPI	Glucose-6-phosphate isomerase	5.3.1.9	1	D	TC
GR	Glutathione reductase	1.6.4.2	1	D	TME
HK	Hexokinase	2.7.1.1	1	D	AC
IDDH	L-Iditol dehydrogenase	1.1.1.14	1	M	TBE
IDHP	Isocitrate dehydrogenase (NADP+)	1.1.1.42	2	D	TME
LAP	Leucine aminopeptidase	3.4.-.-	1	D	TC
LDH	L-Lactate dehydrogenase	1.1.1.27	2	M	TC
αMAN	α-Mannosidase	3.2.1.24	1	D	TBE
MDH	Malate dehydrogenase	1.1.1.37	2	D	AC
MPI	Mannose-6-phosphate isomerase	5.3.1.8	1	D	TC
PEP-GL	Peptidase (glycyl-L-leucine)	3.4.-.-	1	M	TC

Table 2.2. (continued)

Enzyme abbreviation[a]	Enzyme	E.C. no.[b]	Number of loci	Tissue[c]	Buffer[d]
PGK	Phosphoglycerate kinase	2.7.2.3	1	M	TBE
PGM	Phosphoglucomutase	5.4.2.2	1	M	AC
PK	Pyruvate kinase	2.7.1.40	1	D	TME
PNP	Purine-nucleoside phosphorylase	2.4.2.1	1	D	TBE
TAT	Tyrosine aminotransferase	2.6.1.5	1	M	TBE
TPI	Triose-phosphate isomerase	5.3.1.1	1	M	AC
XDH	Xanthine dehydrogenase	1.1.1.204	1	D	TBE

[a]From Shaklee et al. (1990).
[b]Enzyme Commission number.
[c]Tissues used: D, digestive gland; M, muscle.
[d]Buffer systems used: AC, amino-citrate (morpholine) pH 6.9 from Clayton and Tretiak (1972); TC, tris-citrate pH 8.0 from Selander et al. (1971); TBE, tris-borate-EDTA pH 9.1 from Werth (1985); TME, tris-maleate-EDTA pH 7.4 from Selander et al. (1971).

(Nei, 1972), but this assumption is rarely met. Rogers' genetic distance can be used to locate the populations in a Euclidian hyperspace (Wright, 1978). Nei (1987) describes assumptions and limitations of these various distance measures. Nei's genetic distances for all pairwise combinations of collections were used to cluster collections by the unweighted pair-group method with arithmetic means (UPGMA) algorithm (Sneath and Sokal, 1973) using the computer program NTSYS (Rohlf, 1989). Rogers' genetic distance was used to construct two trees "KITSCH" and "FITCH" from the computer program PHYLIP (Felsenstein, 1986). KITSCH and UPGMA assume constant rates of evolution but FITCH does not. For principal component analysis, each allele was treated as a separate variable, with a value equal to the number of copies of the allele (0, 1, or 2) in an individual (Liu et al., 1991). Hierarchical (Weir and Cockerham, 1984) and Wright's (1951, 1965, 1978) F-statistics were calculated for each locus to determine genetic structure within and between collections of Viviparus georgianus and Viviparus goodrichi (see Table 2.3 for the statistical model used). In this study, the three hierarchical levels used were F_{IS}: among individuals within sites as a measure of departure of genotypic frequencies from Hardy-Weinberg expectations; F_{SD}: among sites within drainage systems; F_{DT}: among drainage systems within the total area sampled (Table 2.3).

Nine variables were used for morphological analyses from the 325 electrophoresed snails, plus 30 additional small snails from the Ochlockonee River (O1) to give similar size ranges among the three Viviparus species. Total wet weight (WT) was

Table 2.3. Hierarchical (Weir and Cockerham, 1984) and Wright's (1951, 1978) F-statistics.

Source of variation	Variance component	F-statistics
Hierarchical components		
Drainage system within total sampled area	σ^2_{DT}	$F_{DT} = \sigma^2_{DT} / \sigma^2_{GT}$
Sites within drainage system	σ^2_{SD}	$F_{SD} = \sigma^2_{SD} / (\sigma^2_{GT} - \sigma^2_{DT})$
Individuals within sites	σ^2_{IS}	$F_{IS} = \sigma^2_{IS} / (\sigma^2_{IS} + \sigma^2_{GI})$
Genes within individuals	σ^2_{GI}	
Total	σ^2_{GT}	
Wright's (1951, 1978) components		
Sites within total sampled area	$\sigma^2_{ST} = \sigma^2_{SD} + \sigma^2_{DT}$	$F_{ST} = \sigma^2_{ST} / \sigma^2_{GT}$
Individuals within total sampled area	$\sigma^2_{IT} = \sigma^2_{IS} + \sigma^2_{SD} + \sigma^2_{DT}$	$F_{IT} = \sigma^2_{IT} / \sigma^2_{GT}$

measured at the time of dissection (± 0.001 g). Dry shell weight (WTS) was also recorded later (± 0.001 g). The seven shell measurements [Fig. 2.3: shell height (SH), shell width (SW), two-whorl height (2WH), body whorl height (BWH), second whorl width (2WW), aperture height (AH), and aperture width (AW)] were made using vernier calipers (± 0.1 mm). Number of bands on a shell was not used for a morphological character because recognition of the bands was very difficult for severely eroded, heavily encrusted, and/or dark shells. Before multivariate analyses, the data were $\log_e$-transformed, to reduce the correlation of the measurement means and variances (Sokal and Rohlf, 1981). Moreover, curvilinear relationships in the data were reduced by the $\log_e$-transformation. General size differences among sites and between sexes were tested by a SAS GLM procedure (SAS Institute Inc., 1990) for each species using $\log_e$-transformed total wet weight. Sexual shape dimorphism was examined by principal component analysis.

Three multivariate methods were used to characterize the morphological data: canonical discriminant analysis, which gives maximum discrimination among the groups using linear combinations of variables, discriminant function analysis, which classifies each observation into one of the groups, and principal component analysis, which gives a general overview of the data and tells whether the nine variables are sufficient to discriminate groups (i.e. collections, or sexes). CANDISC, DISCRIM, and PRINCOMP procedures of SAS were used for multivariate analyses (SAS Institute Inc., 1990). Mahalanobis distance (D^2) between the Viviparus species was computed to

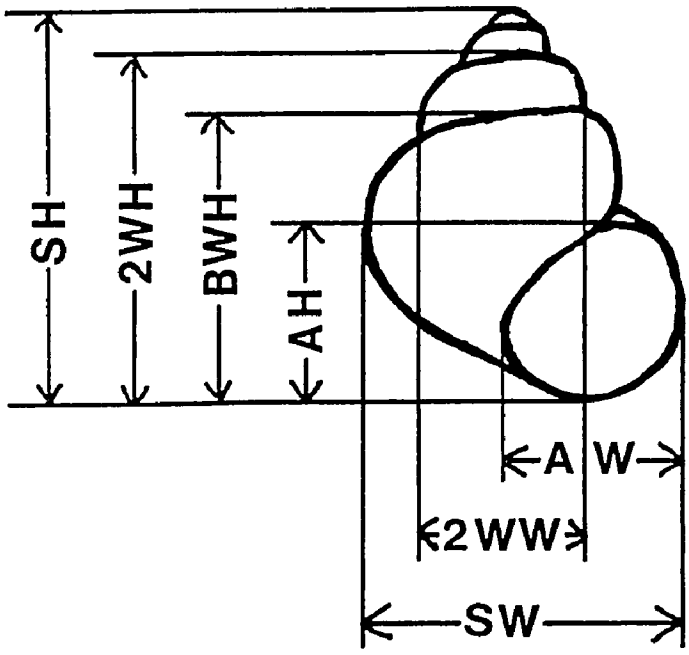


Fig. 2.3. Shell morphology measurements used in this study. Abbreviation: SH, shell height; SW, shell width; 2WH, two-whorl height; BWH, body whorl height; 2WW, second whorl width; AH, aperture height; AW, aperture width.

obtain single numbers which represent the overall morphological differences (Sneath and Sokal, 1973).

Canonical discriminant analysis assumes a common covariance matrix among groups. A likelihood ratio test (Anderson, 1984, Chapter 10) on the within-group covariance matrices showed significant departure from homogeneity. Therefore, within-group covariance matrices rather than the pooled covariance matrix were used for the discriminant function analysis. If the lack of homogeneity affects group configuration significantly, canonical discriminant analysis is not reliable. However, the discriminant function analysis (which is similar in principle to canonical discriminant analysis [James and McCulloch, 1990]), gave similar misclassification proportions when based on either a pooled covariance matrix (4.5% misclassification) or on separate within-group covariance matrices (2.8%). Therefore, use of a pooled covariance matrix should not affect graphical presentation of the canonical discriminant analysis, and results of the canonical discriminant analysis are valid.

For the principal component analysis, principal components were calculated from a covariance matrix because the first principal component scores can be used to obtain bivariate allometric equations (Jolicoeur, 1963). Although size and shape are often confounded in the first two principal components in a multigroup analysis (Humphries et al., 1981), my data did not show the confounding such as apparent positive correlation between the first and second principal components for each group (collection). Therefore, individuals were compared based on several principal component scores without adjustment for body

size. For the morphological principal component analysis, if the eigenvector values for the first principal component are all positive, the first principal component is termed the "size" component and the others are termed the "shape" component (Marcus, 1990).

Results

Genetics

The eleven collections of the Viviparus georgianus complex were separated by repeated fixed allele differences (Fig. 2.4) into three allopatric species: a western species (Viviparus goodrichi), the Ochlockonee River species Viviparus limi, and an eastern species (Viviparus georgianus). The Ochlockonee River species (O1) was located between the western (C1, A1, A2, and A3) and eastern (S1, S2, W1, J1, J2, and J3) species. Allele frequencies at each of the 27 polymorphic loci for each collection site are given in Table 2.4; the 11 completely monomorphic loci (*AH, ALP, AO, ESTD, FH, αGLUS, G6PDH, LDH-2, αMAN, MDH-1,* and *MPI*) are excluded. There are 11 diagnostic loci, 9 of which are fixed for different alleles, between V. goodrichi and V. limi (Fig. 2.4). Fourteen diagnostic loci (including 7 loci fixed for different alleles) separate V. limi and V. georgianus. Seven diagnostic loci (including 5 loci fixed for different alleles) separate V. goodrichi and V. georgianus.

Principal component analysis of all polymorphic loci clearly separated the 11 populations into three distinct species. Of the total genetic variation in the data set, the

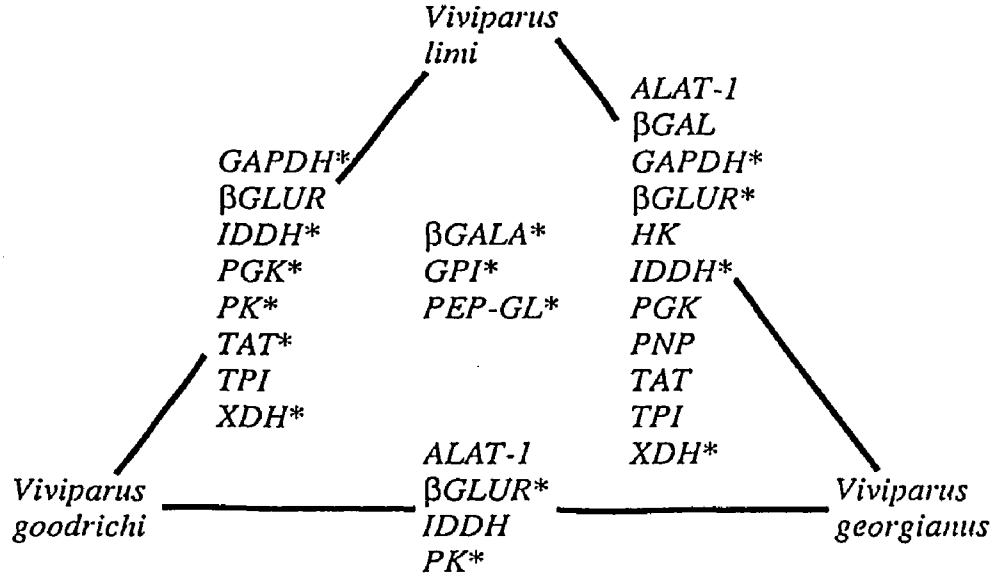


Fig. 2.4. Diagnostic allozyme loci for the three Viviparus species. The three middle loci are diagnostic for the three species. *: fixed allele differences between species.

Table 2.4. Allele frequencies and heterozygosities for 27 polymorphic loci in 11 collections of the Viviparus georgianus complex. Number of individuals sampled are in Table 2.1.

Locus	Allele[a]	V. goodrichi				V. limi	V. georgianus					
		C1[b]	A1	A2	A3	O1	S1	S2	W1	J1	J2	J3
AAT-1	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00
	47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
	H_o[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
	H_e[d]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
AAT-2	100	1.00	1.00	1.00	1.00	0.50	1.00	1.00	1.00	1.00	1.00	1.00
	76	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.53	0.00	0.00	0.00	0.00	0.00	0.00
	H_e	0.00	0.00	0.00	0.00	0.51	0.00	0.00	0.00	0.00	0.00	0.00
AK	104	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00
	100	1.00	1.00	1.00	1.00	1.00	0.20	1.00	1.00	1.00	1.00	1.00
	94	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.00	0.00
	H_e	0.00	0.00	0.00	0.00	0.00	0.51	0.00	0.00	0.00	0.00	0.00
ALAT-1	144	0.20	0.02	0.07	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	128	0.73	0.98	0.93	0.96	0.98	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.07	0.00	0.00	0.02	0.02	1.00	1.00	1.00	1.00	1.00	1.00
	H_o	0.27	0.03	0.13	0.07	0.03	0.00	0.00	0.00	0.00	0.00	0.00
	H_e	0.42	0.03	0.13	0.07	0.03	0.00	0.00	0.00	0.00	0.00	0.00

Table 2.4. (continued)

Locus	Allele	*V. goodrichi*				*V. limi*	*V. georgianus*					
		C1	A1	A2	A3	O1	S1	S2	W1	J1	J2	J3
ARK	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00
	63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
	H_e	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
βGAL	112	0.00	0.08	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	106	1.00	0.75	0.63	1.00	1.00	0.00	0.00	0.00	0.08	0.00	0.00
	100	0.00	0.17	0.00	0.00	0.00	1.00	0.88	1.00	0.92	0.60	1.00
	95	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.40	0.00
	H_o	0.00	0.37	0.40	0.00	0.00	0.00	0.23	0.00	0.10	0.19	0.00
	H_e	0.00	0.41	0.47	0.00	0.00	0.00	0.21	0.00	0.16	0.49	0.00
βGALA	100	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
	98	1.00	1.00	0.97	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	96	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	88	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	H_o	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	H_e	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GAPDH	100	1.00	1.00	1.00	1.00	0.00	0.98	1.00	1.00	1.00	0.98	1.00
	74	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02	0.00
	63	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.04	0.00
	H_e	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.04	0.00

Table 2.4. (continued)

Locus	Allele	*V. goodrichi*				*V. limi*	*V. georgianus*					
		C1	A1	A2	A3	O1	S1	S2	W1	J1	J2	J3
βGLUR	131	1.00	1.00	0.98	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	107	0.00	0.00	0.02	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.98	1.00	0.92	1.00
	80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.08	0.00
	H_o	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.03	0.00	0.15	0.00
	H_e	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.03	0.00	0.14	0.00
G3PDH	109	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.90	0.95	0.88	0.90	0.98	0.57	0.00	0.00	0.55	0.83	0.15
	88	0.10	0.00	0.12	0.10	0.02	0.43	1.00	1.00	0.45	0.17	0.85
	H_o	0.20	0.10	0.23	0.20	0.03	0.40	0.00	0.00	0.43	0.35	0.23
	H_e	0.18	0.10	0.21	0.18	0.03	0.50	0.00	0.00	0.50	0.29	0.26
GPI	102	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
	86	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GR	100	1.00	1.00	1.00	1.00	0.98	0.53	1.00	1.00	1.00	1.00	1.00
	89	0.00	0.00	0.00	0.00	0.02	0.47	0.00	0.00	0.00	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.03	0.53	0.00	0.00	0.00	0.00	0.00
	H_e	0.00	0.00	0.00	0.00	0.03	0.51	0.00	0.00	0.00	0.00	0.00

Table 2.4. (continued)

Locus	Allele	*V. goodrichi*				*V. limi*	*V. georgianus*					
		C1	A1	A2	A3	O1	S1	S2	W1	J1	J2	J3
HK	100	0.00	0.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	0.98	1.00
	96	1.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.02	0.00
	H_o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
	H_e	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
IDDH	279	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	160	0.00	0.00	0.00	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.00	0.00	0.00	0.00	0.00	0.98	0.93	0.97	1.00	1.00	1.00
	53	1.00	1.00	1.00	1.00	0.00	0.02	0.07	0.03	0.00	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.03	0.03	0.13	0.07	0.00	0.00	0.00
	H_e	0.00	0.00	0.00	0.00	0.03	0.03	0.13	0.07	0.00	0.00	0.00
IDHP-1	128	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	H_o	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	H_e	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
IDHP-2	100	1.00	1.00	1.00	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00
	20	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00
	H_e	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00

Table 2.4. (continued)

Locus	Allele	V. goodrichi				V. limi	V. georgianus					
		C1	A1	A2	A3	O1	S1	S2	W1	J1	J2	J3
LAP-1	106	0.07	0.00	0.00	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.93	1.00	1.00	1.00	0.68	1.00	1.00	1.00	1.00	1.00	1.00
	H_o	0.13	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.00	0.00	0.00
	H_e	0.13	0.00	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00
LDH-1	112	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	100	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00
	H_o	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
	H_e	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
MDH-2	100	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	60	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	H_o	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	H_e	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PEP-GL	104	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	103	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
PGK	127	0.00	0.00	0.00	0.00	1.00	0.10	0.00	0.02	0.00	0.08	0.08
	100	1.00	1.00	1.00	1.00	0.00	0.90	1.00	0.98	1.00	0.92	0.92
	H_o	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.03	0.00	0.15	0.10
	H_e	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.03	0.00	0.14	0.16

Table 2.4. (continued)

Locus	Allele	V. goodrichi				V. limi	V. georgianus					
		C1	A1	A2	A3	O1	S1	S2	W1	J1	J2	J3
PGM	107	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	1.00	1.00
	56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
	H_e	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
PK	118	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PNP	100	0.10	0.00	0.00	1.00	0.02	0.70	1.00	1.00	1.00	1.00	1.00
	81	0.90	1.00	0.95	0.00	0.98	0.30	0.00	0.00	0.00	0.00	0.00
	62	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	H_o	0.20	0.00	0.03	0.00	0.03	0.47	0.00	0.00	0.00	0.00	0.00
	H_e	0.18	0.00	0.10	0.00	0.03	0.43	0.00	0.00	0.00	0.00	0.00
TAT	100	1.00	1.00	1.00	1.00	0.00	1.00	0.98	1.00	1.00	1.00	1.00
	93	0.00	0.00	0.00	0.00	1.00	0.00	0.02	0.00	0.00	0.00	0.00
	H_o	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
	H_e	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
TPI	100	1.00	1.00	1.00	0.97	0.00	0.97	0.93	0.97	0.86	0.96	0.97
	6	0.00	0.00	0.00	0.03	1.00	0.03	0.07	0.03	0.13	0.04	0.03
	H_o	0.00	0.00	0.00	0.07	0.00	0.07	0.07	0.07	0.20	0.08	0.07
	H_e	0.00	0.00	0.00	0.07	0.00	0.07	0.13	0.07	0.24	0.08	0.07

Table 2.4. (concluded)

Locus	Allele	V. goodrichi				V. limi	V. georgianus					
		C1	A1	A2	A3	O1	S1	S2	W1	J1	J2	J3
XDH	110	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	100	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
Mean H_o (%)		3.07	1.32	2.63	0.88	2.98	5.53	1.58	0.54	2.37	2.73	1.05

[a]Numbers represent relative allelic mobility.
[b]See Table 2.1 for site abbreviations.
[c]Observed heterozygosity.
[d]Expected heterozygosity.

first to the sixth principal components accounted for 30%, 22%, 5%, 4%, 3%, and 3%, respectively (67% in all). The major grouping was due to the first and second principal component scores, which primarily reflected variation at the diagnostic loci listed in Fig. 2.4 and also the polymorphic loci *AAT-2*, *G3PDH*, and *LAP-1*. Only the first and second principal component scores were plotted (Fig. 2.5). The first and second principal components clearly separated all three species. There was no evidence for hybrid individuals or for introgression among the species, out of 325 snails electrophoresed. The third principal component reflected minor intraspecific variation in the *AK* and *GR* loci between site S1 and the other populations of the eastern species.

Nei's genetic distance (D_N) and Rogers' genetic distance (D_R) are listed in Table 2.5. The genetic distances among species were large when measured by either method (D_N, 0.230-0.517; D_R, 15.3-29.8) but the within-species differences were small (D_N, 0.000-0.057; D_R, 0.0-4.2). Moreover, the genetic distances (both D_N and D_R) between V. limi and the other two species were larger than genetic distances between the western species (Viviparus goodrichi) and the eastern species (Viviparus georgianus), although the Ochlockonee River species (Viviparus limi) is distributed between the eastern and western species. Therefore, a UPGMA analysis of D_N clustered within-species locations first, then V. goodrichi and V. georgianus were clustered, and V. limi were separated last (Fig. 2.6). The clustering results from the other two methods, KITSCH and FITCH,

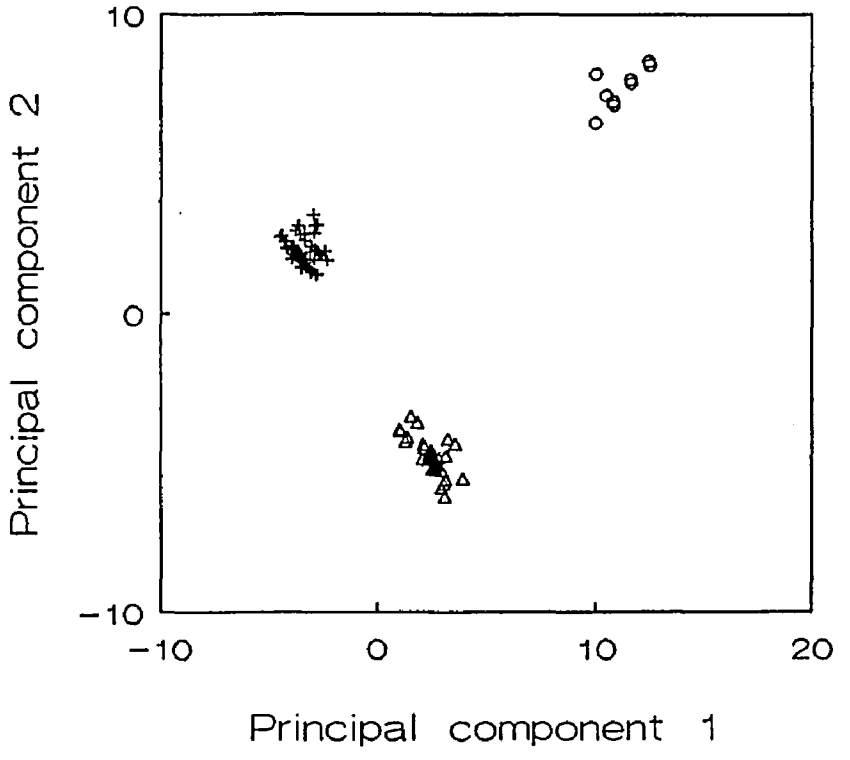


Fig. 2.5. Principal component scores of genetic data for the three Viviparus species (△: V. goodrichi; ○: V. limi; +: V. georgianus).

Table 2.5. Nei's (1978) genetic distance (above diagonal) and modified Rogers' (Wright, 1978) genetic distance (below diagonal) for 11 populations of the Viviparus georgianus complex. Site abbreviations as in Table 2.1.

Sites	C1	A1	A2	A3	O1	S1	S2	W1	J1	J2	J3
C1	-	0.003	0.005	0.051	0.369	0.320	0.319	0.322	0.299	0.288	0.318
A1	0.28	-	0.002	0.056	0.365	0.319	0.324	0.326	0.301	0.290	0.321
A2	0.40	0.16	-	0.057	0.370	0.319	0.319	0.323	0.301	0.289	0.318
A3	3.76	4.12	4.19	-	0.438	0.276	0.258	0.261	0.238	0.230	0.257
O1	22.76	22.77	22.84	26.49	-	0.504	0.513	0.517	0.478	0.465	0.504
S1	19.93	20.04	19.93	17.80	28.81	-	0.033	0.032	0.024	0.030	0.028
S2	20.29	20.73	20.34	17.07	29.82	2.42	-	0.000	0.009	0.021	0.001
W1	20.57	20.97	20.62	17.35	30.17	2.38	0.04	-	0.008	0.023	0.001
J1	19.08	19.39	19.23	15.85	28.10	1.78	0.66	0.65	-	0.006	0.005
J2	18.44	18.73	18.54	15.30	27.40	2.19	1.58	1.71	0.47	-	0.017
J3	20.25	20.61	20.32	17.04	29.47	2.07	0.11	0.06	0.37	1.26	-

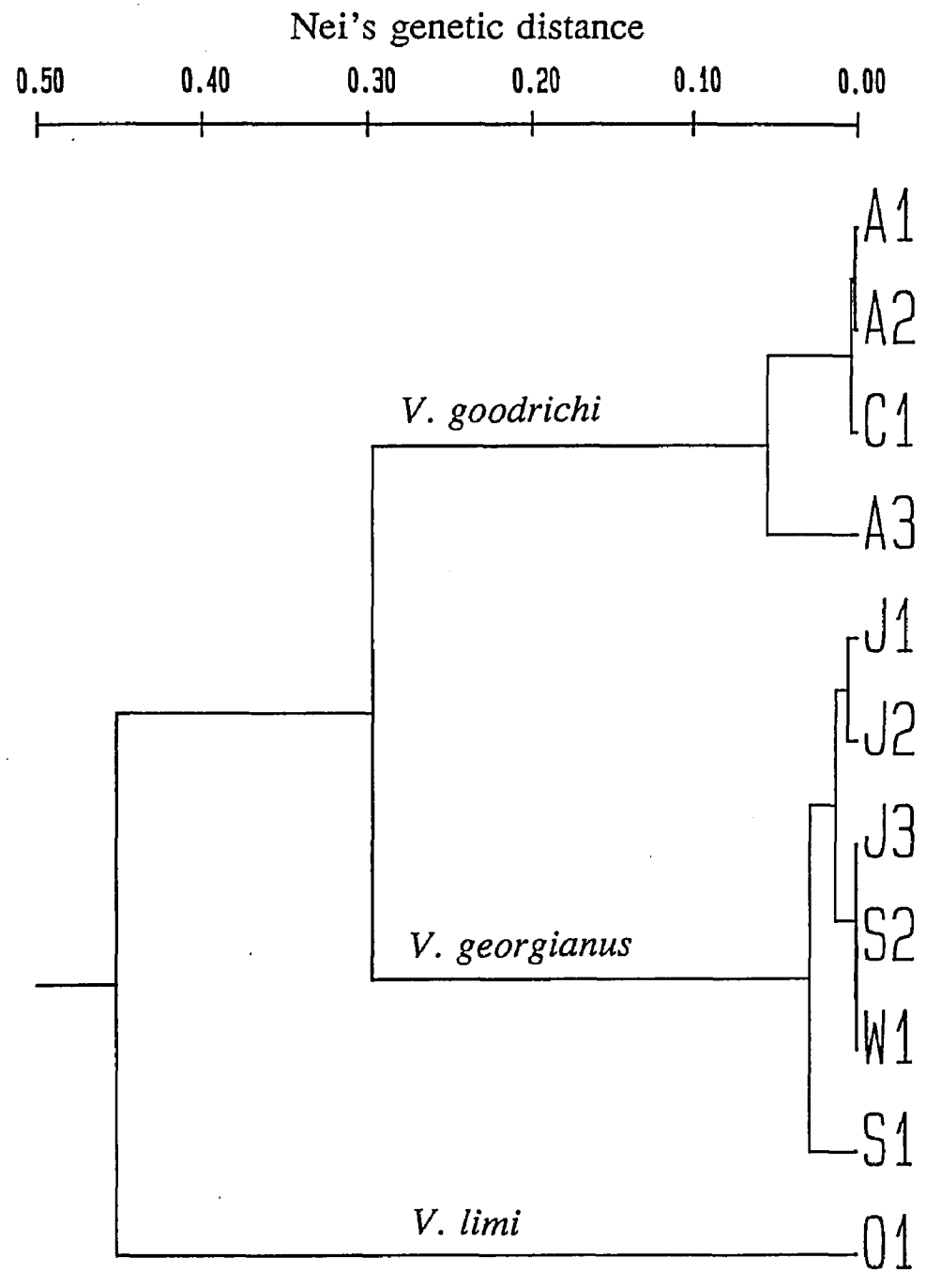


Fig. 2.6. UPGMA phenogram for 11 populations of the Viviparus georgianus complex based on Nei's (1978) genetic distance. Homes River (C1), Chipola River in Jackson Co. (A1), Chipola River in Calhoun Co. (A2), Spring Creek (A3), Lake Talquin (O1), Santa Fe River (S1), Withlacoochee River of Suwannee (S2), Withlacoochee River in southern Florida (W1), Silver River (J1), Wekiva River (J2), and Lake Monroe (J3).

which are not shown, agreed with the UPGMA result concerning the three major grouping (species).

Genetic structure within and among collections of Viviparus goodrichi and Viviparus georgianus was determined by the hierarchical statistics F_{IS}, F_{SD}, and F_{DT}, calculated for the 12 and 17 loci that were polymorphic in one or more collections, respectively (Table 2.6). Because of fixed or nearly fixed allele differences within drainage systems, F_{SD} was large for both species. The negative values of F_{DT} indicate that the estimate of the mean square of the higher level (among drainage systems) was less than the estimate of the mean square of the lower level (within drainage systems) because there was no added variance component among drainage systems (Sokal and Rohlf, 1981, p. 214). These statistics indicate that most of the intraspecific variation was within drainage system differences, not between drainage system differences. Low dispersal within a river is just as important as isolation between rivers in producing genetic differentiation. The mean F_{IS} values indicated that 7 and 11% deficiencies of heterozygotes were observed (compared to Hardy-Weinberg proportions) in Viviparus goodrichi and Viviparus georgianus, respectively.

Morphology

Mean size ($\log_e$-transformed total wet weight) of the snails was highly significantly different between the sexes in Viviparus limi (Table 2.7, $p < 0.0001$), nominally significantly different in Viviparus georgianus ($p < 0.05$), and not significantly different in Viviparus goodrichi ($p > 0.05$).

Table 2.6. Jacknifed means (standard errors) of hierarchical F-statistics from Weir and Cockerham (1984) for Viviparus goodrichi and V. georgianus.

	Viviparus goodrichi	Viviparus georgianus
F_{DT}	-0.336(0.143)	-0.049(0.020)
F_{SD}	0.519(0.127)	0.387(0.082)
F_{ST}	0.339(0.085)	0.356(0.086)
F_{IT}	0.728(0.185)	0.480(0.075)
F_{IS}	0.073(0.064)	0.112(0.049)

Table 2.7. ANOVA tests from type III SS for sexual dimorphism of *Viviparus* based on $\log_e$-transformed total wet weight.

Species	Source of Variation	Degrees of freedom	Mean square	F
V. goodrichi	Locality	3	18.513	80.91***
	Sex	1	0.071	0.31
	Error	115	0.229	
V. limi	Sex	1	15.187	42.84***
	Error	58	0.355	
V. georgianus	Locality	5	9.579	79.18***
	Sex	1	0.480	3.97*
	Error	168	0.121	

*P < 0.05

***P < 0.0001

Since the three independent tests were conducted simultaneously, the nominally significant result for V. georgianus was not significant when a Bonferroni adjustment was made. Females were 2.84 and 1.26 times larger than males in V. limi and V. georgianus, respectively, based on means of total wet weight (no transformation). There were significant site effects on size in V. georgianus and V. goodrichi (both $p < 0.0001$), which were represented by multiple collections (Table 2.7). There was no apparent shape differences between sexes, based on principal component scores (not shown).

Canonical discriminant analysis using nine variables separated the three species clearly with little overlap (Fig. 2.7). The first canonical variable separated Viviparus goodrichi from the other two species, yet did not discriminate V. georgianus and V. limi (Fig. 2.7). The first canonical variable had very high negative correlations with aperture height, aperture width, and shell width, and a high positive correlation with shell weight (Table 2.8). V. goodrichi had a lighter, more globose shell with a larger aperture than V. georgianus and V. limi. The remaining two species, V. georgianus and V. limi, were separated by the second canonical variable with little overlap (Fig. 2.7). The second canonical variable had high negative correlations with aperture width, shell width, second whorl width, and shell weight (Table 2.8). V. georgianus was globose with a circular aperture and V. limi was more elongate and lighter with a longer aperture height.

Although the three Viviparus species were morphologically distinguishable by canonical discriminant analysis, the pattern

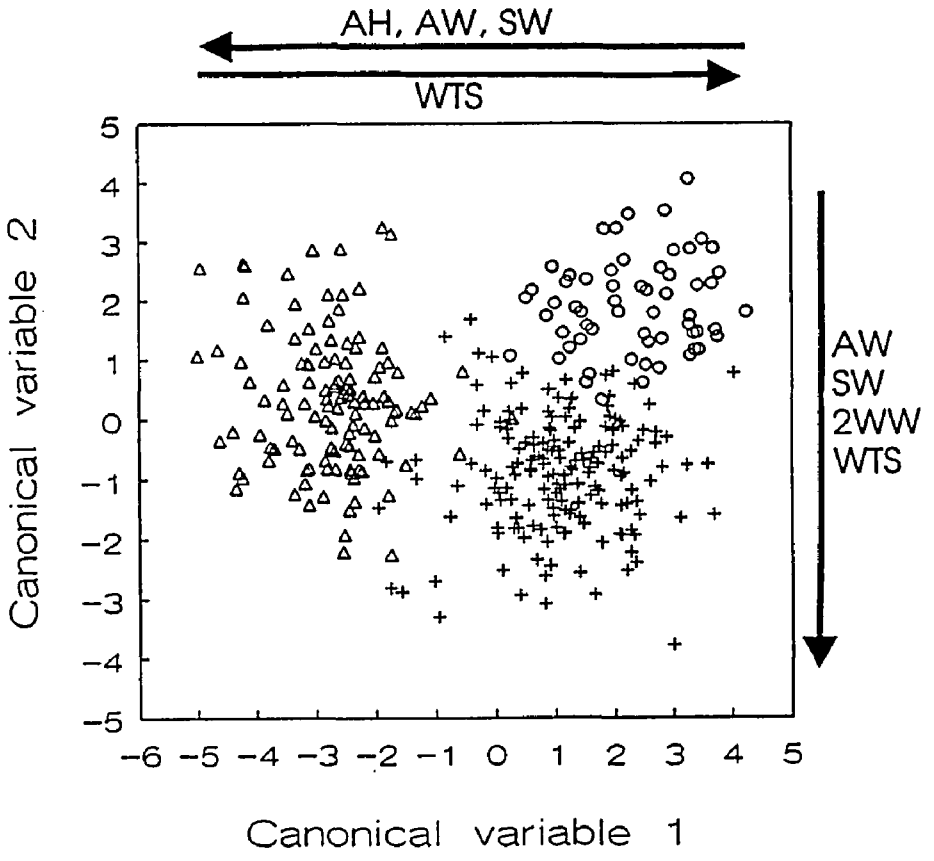


Fig. 2.7. Separation of the three Viviparus species (△: V. goodrichi; ○: V. limi; +: V. georgianus) by the first and second canonical variables of nine morphological measurements.

Table 2.8. Total canonical structure of nine morphological variables for three Viviparus species.

Variable	First Canonical Variable	Second Canonical Variable
Total weight	-0.027	-0.087
Shell weight	0.190	-0.124
Shell height	-0.043	-0.010
Shell width	-0.213	-0.140
Two-whorl height	-0.078	-0.026
Body whorl height	-0.125	-0.047
Second whorl width	-0.142	-0.139
Aperture height	-0.312	0.002
Aperture width	-0.260	-0.145

of morphological differentiation did not agree with the pattern of genetic differentiation. The smallest Mahalanobis distance (D^2) based on the morphological data was between V. limi and V. georgianus (9.4). The distance between V. limi and V. goodrichi was 28.1, and between V. goodrichi and V. georgianus, 15.1. Based on the genetic distance measurements, V. goodrichi and V. georgianus were more closely related (Table 2.5).

Discriminant function analysis classified most of the snails into the correct species (Table 2.9). Only ten snails (2.8%) were wrongly classified out of 355 snails studied. No snails were misclassified between Viviparus goodrichi and V. limi (Table 2.9).

Principal component analysis of each species revealed intraspecific variation. Since size was highly variable (Table 2.10), the first principal component, which reflected size differences, explained most (98.0-99.2%) of the total morphological variation within each species; the second and third principal components, which reflected shape differences, accounted for very small additional percentages 0.5-1.4% and 0.1-0.3%, respectively (Table 2.11). In Viviparus goodrichi, the Chipola River population (A2) had lower second principal component scores than the other collections (Fig. 2.8, A). The second principal component was characterized by a large negative element of eigenvector for shell weight (Table 2.11), which means the Chipola River population (A2) had heavier shells. In V. georgianus, the Wekiva River (J2) and Lake Monroe (J3) populations had high second principal component scores compared to the Suwannee River populations (S1 and S2; Fig. 2.8, B). The

Table 2.9. Discriminant function analysis for three Viviparus species based on nine morphological variables.

From species	Number of individuals (%) classified into species						
	V. goodrichi		V. limi		V. georgianus		Total
V. goodrichi	119	(99.2)	0	(0.0)	1	(0.8)	120 (100.0)
V. limi	0	(0.0)	59	(98.3)	1	(1.7)	60 (100.0)
V. georgianus	6	(3.4)	2	(1.1)	167	(95.5)	175 (100.0)
Total	125		61		169		355

Table 2.10. Means (standard deviations) of total wet weight (WT), shell weight (WTS) and 7 shell measurements (mm) for 11 populations of three Viviparus species. Abbreviations: SH, Shell height; SW, shell width; 2WH, two-whorl height; BWH, body whorl height; 2WW, second whorl width; AH, aperture height; AW, aperture width. (see Table 2.1 for sample sizes).

Sites or species	WT (g)	WTS (g)	SH (mm)	SW (mm)	2WH (mm)	BWH (mm)	2WW (mm)	AH (mm)	AW (mm)
V. goodrichi									
Total	4.92(3.44)	1.64(1.06)	25.6(6.94)	22.3(5.36)	24.3(6.44)	22.1(5.78)	16.8(4.38)	17.0(3.71)	13.5(3.04)
C1	5.97(1.29)	1.89(0.47)	28.7(1.97)	25.2(1.63)	27.7(1.87)	25.2(1.64)	19.3(1.38)	18.9(1.03)	14.9(1.03)
A1	1.22(0.51)	0.43(0.15)	16.8(2.39)	15.3(1.91)	15.9(2.18)	14.5(1.91)	11.2(1.45)	12.0(1.66)	9.6(1.20)
A2	5.58(3.20)	2.13(1.03)	27.2(5.78)	23.6(4.32)	25.6(5.24)	23.4(4.57)	17.7(3.35)	18.2(2.96)	14.5(2.47)
A3	6.93(4.06)	2.11(1.12)	29.9(6.54)	25.1(4.93)	28.0(5.80)	25.4(5.22)	19.2(4.17)	19.1(2.87)	15.2(2.79)
V. limi									
O1	4.21(2.72)	1.89(1.07)	24.9(6.63)	19.2(4.36)	23.0(5.86)	20.3(4.92)	14.9(3.79)	14.6(3.05)	11.5(2.51)
V. georgianus									
Total	4.48(2.47)	2.01(1.09)	24.9(5.80)	20.8(3.98)	23.3(5.06)	20.9(4.29)	16.1(3.29)	15.0(2.64)	12.5(2.17)
S1	5.87(2.27)	2.86(1.12)	27.0(4.39)	23.2(3.29)	25.9(4.15)	23.4(3.50)	17.6(2.62)	16.5(1.85)	13.9(1.82)
S2	6.19(0.99)	2.96(0.43)	27.6(1.52)	23.4(1.23)	26.4(1.46)	23.8(1.31)	17.9(1.14)	17.5(0.91)	14.1(0.79)
W1	2.28(0.65)	1.10(0.28)	20.2(2.37)	17.0(1.73)	18.8(2.14)	17.0(1.76)	13.1(1.43)	12.3(1.03)	10.4(0.93)
J1	2.35(1.04)	1.08(0.39)	20.2(2.89)	17.4(2.23)	18.9(2.55)	17.0(2.16)	13.4(1.93)	12.7(1.36)	10.5(1.19)
J2	2.51(1.07)	1.04(0.39)	20.2(3.09)	18.1(2.65)	19.4(2.89)	17.7(2.62)	13.7(2.17)	13.3(1.60)	11.2(1.47)
J3	7.35(1.22)	2.89(0.48)	33.5(2.05)	25.4(1.28)	29.8(1.60)	26.0(1.32)	20.4(1.15)	17.7(0.75)	14.7(0.62)

Table 2.11. Eigenvectors and eigenvalues of principal components for three *Viviparus* species based on nine morphological variables.

	V. goodrichi		*V. limi*	*V. georgianus*		
Variables	PRIN1	PRIN2	PRIN1	PRIN1	PRIN2	PRIN3
Total weight	0.624	0.343	0.629	0.618	0.226	-0.036
Shell weight	0.580	-0.802	0.554	0.582	-0.764	-0.108
Shell height	0.214	0.240	0.228	0.227	0.338	-0.493
Shell width	0.190	0.181	0.195	0.192	0.209	0.245
Two-whorl height	0.211	0.216	0.219	0.218	0.233	-0.121
Body whorl height	0.209	0.187	0.210	0.207	0.193	0.105
Second whorl width	0.207	0.214	0.217	0.203	0.310	-0.193
Aperture height	0.175	0.106	0.179	0.174	0.078	0.570
Aperture width	0.175	0.101	0.189	0.173	0.119	0.544
Eigenvalue	1.758	0.015	1.529	1.030	0.014	0.003
% of total variance	98.81	0.82	99.22	98.04	1.36	0.28

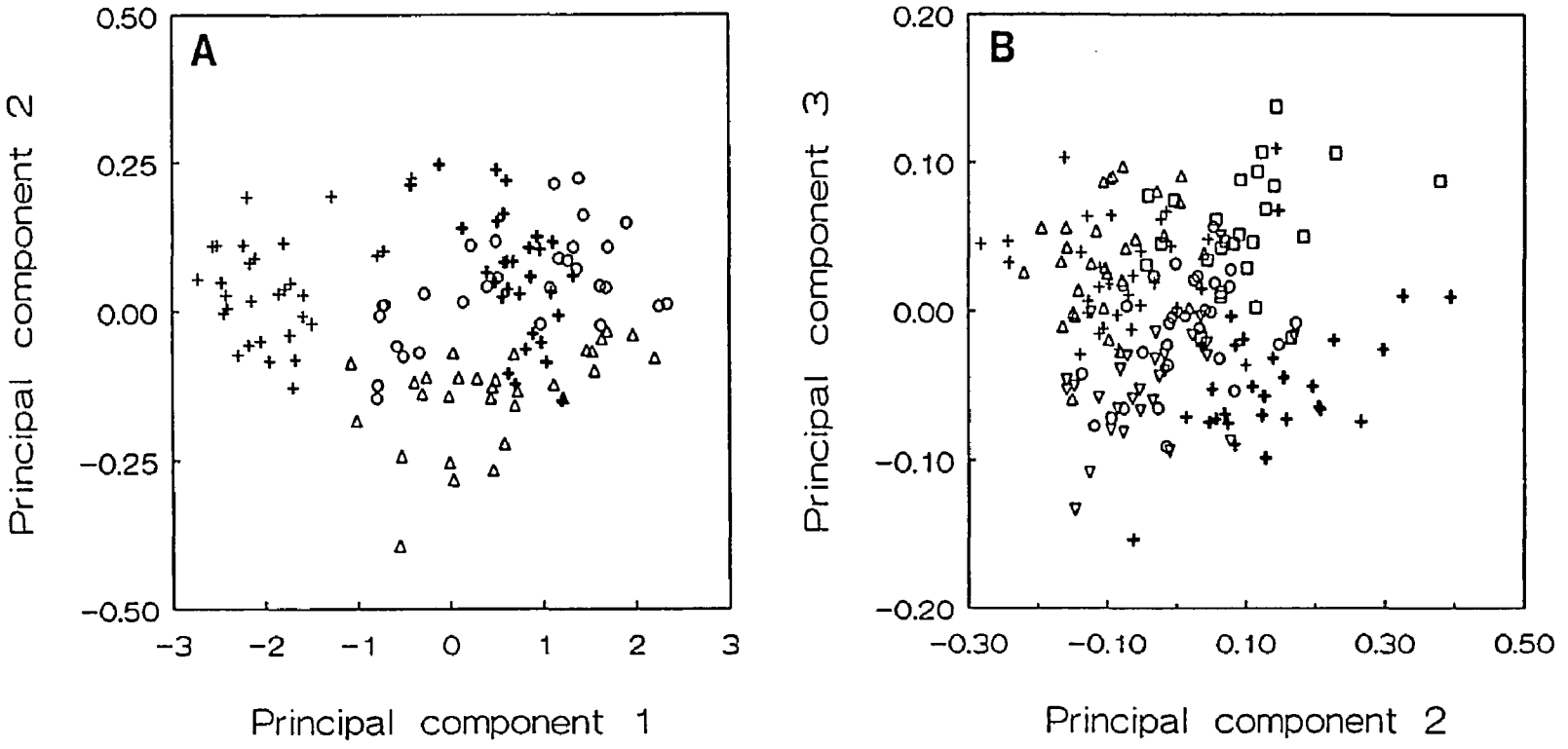


Fig. 2.8. Intraspecific morphological variation of two Viviparus species based on principal component scores. (A) V. goodrichi: +, Homes River (C1); +, Chipola River in Jackson Co. (A1); ▵, Chipola River in Calhoun Co. (A2); o, Spring Creek (A3). (B) V. georgianus: +, Santa Fe River (S1); ▵, Withlacoochee River of Suwannee (S2); ▽, Withlacoochee River in southern Florida (W1); o, Silver River (J1); □, Wekiva River (J2); +, Lake Monroe (J3).

second principal component was characterized by a large negative element of eigenvector for shell weight and a large positive element for shell height. The Wekiva River (J2) and Lake Monroe (J3) populations had long, light shells and the Suwannee River populations (S1 and S2) had short, heavy shells, due to the eroded apex of snails collected at sites S1 and S2 (Fig. 2.1). The Wekiva River (J2) and Lake Monroe (J3) populations were separated by the third principal component (Fig. 2.8, B). The third principal component had large positive elements of eigenvectors for aperture height and aperture width, and a large negative element for shell height (Table 2.11), which means the Lake Monroe population (J3) is characterized by individuals with longer shells with smaller apertures than the Wekiva River population (J2).

Discussion

Genetic subdivision

These eleven populations of the Viviparus georgianus complex in the southeastern United States became separated into three genetically distinct groups (species) by multiple gene substitutions and consequent large interspecific genetic distances. Each drainage system supported only one of the three species (i.e. the three Viviparus species were allopatric). I applied the phylogenetic species concept (Wiley, 1981) to the allopatric snail populations in this study instead of the traditional biological species concept (Mayr, 1942, 1963, 1969, 1970) because reproductive isolation is difficult to measure for allopatric populations and is inconsistently related to

phenotypic and genotypic differentiation (McKitrick and Zink, 1988). The phylogenetic species concept defines a species as a lineage which maintains its genetic integrity from other such lineages and do not concern reproductive compatibilities directly (Wiley, 1981). On the contrary, the biological species concept, which focuses on reproductive isolation and its intrinsic mechanisms, is not practical for allopatric populations.

Additional morphological and ecological reasons also support the recognition of these species: (1) the three groups showed morphological differences, (2) only Viviparus limi showed clear sexual dimorphism in size, which may imply life-history differences (such as in life-span between sexes). Life history differences between sexes and sexual size dimorphism have been reported for Viviparus georgianus populations in New York and Connecticut (Van Cleave and Lederer, 1932; Browne, 1978; Jokinen et al., 1982). On the other hand, V. georgianus in Florida (probably V. goodrichi) did not show sexual size differences (Vail, 1978).

Although intraspecific differentiation was less pronounced, V. goodrichi populations were subdivided within the Apalachicola River system based on one fixed and another nearly fixed locus for different alleles, and V. georgianus populations were subdivided within the Suwannee and St. Johns river systems based on large allele frequency differences at four loci. Interspecific allopatry may be explained by a land barrier to migration (or gene flow) among species, strong competitive exclusion, strong differential selection, or combinations of the

above forces. Such significant intraspecific variation suggests existence of genetic barriers within a continuous drainage system or strong selection.

Large intraspecific variation within the same drainage systems was reflected in large F_{SD} values in Viviparus goodrichi and V. georgianus populations (Table 2.6). This result indicates that gene flow among snails in the different tributaries is limited. Reasons for the limited gene flow among connected tributaries within a single drainage system are not clear, yet several possible explanations exist. First, unbalanced gene flow between upstream and downstream populations may cause genetic differentiation among tributaries. For example, Dillon (1988) transplanted freshwater snails which possessed a different allozyme allele from native conspecific snails. Later collection indicated that the unique allele was spread more upstream than downstream through migration and hybridization (Dillon, 1988). The result indicated that upstream gene flow was higher than downstream, which keeps headwater populations from being mixed. The upstream gene flow can be explained by rheotaxis of viviparid snails (Bovbjerg, 1952). Conversely, limited gene flow from downstream to upstream due to water currents could cause genetic separation among tributaries. Secondly, an uninhabitable area such as a large river between tributaries could be a genetic barrier for some species since Viviparus are absent from large rivers (Clench and Turner, 1956).

Wright's (1951, 1965, 1978) F statistics are measures of genetic differentiation in populations. Freshwater Viviparus

snails seem to have high F_{ST} values (F_{ST} = 0.338 for V. georgianus, F_{ST} = 0.505 for V. goodrichi; this study). F_{ST} is a measure of genetic differentiation among subpopulations (e.g., collections or sites) within the total species range, without recognizing any intermediate hierarchical levels, and varies between 0 and 1. Low values mean that populations are homogeneous in allele frequencies and suggest that there is (1) high gene flow among them, (2) low allele frequency variance due to large effective population size, (3) strong uniform selection, or (4) combinations of these. The F_{ST} for the Viviparus snails are higher than marine prosobranchs with pelagic larvae. This result indicates prevalent gene flow in marine environments, large population size, or strong uniform selection (e.g., F_{ST} = 0.018 and 0.004 for two species in the Stramonita haemastoma complex, Liu et al., 1991; F_{ST} = 0.021 for Littorina littorea, Janson, 1987). Marine prosobranchs, which lack pelagic larvae and are thus comparable to the Viviparus snails in dispersal potential, seem to have more restricted gene flow or different strength of selection. Consequently, they show higher F_{ST} values (e.g., F_{ST} = 0.201 for Nucella emarginata, Palmer et al., 1990; F_{ST} = 0.078 for Littorina saxatilis, Janson, 1987) than marine prosobranchs with pelagic larvae, but still did not have as high F_{ST} values as the freshwater Viviparus prosobranchs. The higher F_{ST} values in the Viviparus snails suggests that factors other than larval dispersal (such as geographical barriers) increase subdivision in freshwater systems.

Besides large interspecific and intraspecific variation, allele frequencies were similar in multiple drainage systems

(sites C1, A1, and A2 in Viviparus goodrichi and sites S2, W1, and J3 in V. georgianus). This similarity could be due to historical gene flow caused by river capture and flooding. For example, the Apalachicola River used to flow into the Choctawhachee River (Puri and Vernon, 1964), which may explain the genetic similarity between these river systems for V. goodrichi.

Biogeography

Viviparus goodrichi and Viviparus georgianus are an invertebrate example of east-west genetic discontinuity in the southeastern United States, similar to that reported previously in vertebrates (e.g., Avise et al., 1979; Bermingham and Avise, 1986). Concordant findings of east-west breaks indicate that a shared geologic history determines genetic structure of populations. Vicariance biogeographic models thus seem to be best for explaining patterns of genetic variation in the southeastern United States.

However, the position of the discontinuity is different among the groups of species or the populations within a species. The most westerly border was reported in Lepomis gulosus between the Alabama/Tombigbee and Escambia rivers and the most easterly border was in Lepomis punctatus between the Apalachicola and Suwannee rivers (Bermingham and Avise, 1986). These differences suggest that vicariance events did not separate all species in the same way, or dispersal after vicariance caused distributional differences among species.

A major reason for the east-west genetic break is thought to be the interglacial rise in sea level (Neill, 1957; Bermingham and Avise, 1986). Since the Apalachicola drainage system was embayed farther inland than any other at each rise of sea level, the salt water channel that occupied that position during long periods of the Pleistocene seems to be a very important barrier for many organisms (Neill, 1957). Hence, freshwater organisms appeared to be separated not only by land but also by salt water channels in the major drainage system caused by sea level rises.

Besides the finding of an east-west genetic discontinuity, some surprising results were obtained: the Ochlockonee River species Viviparus limi, which occurs between the eastern species V. goodrichi and the western species V. georgianus, was allozymically detected and very different from them. Three other endemic species (Campeloma parthenum [Vail, 1979a], Lioplax talquinensis [Vail, 1979b], and Alasmidonta wrightiana [Walker, 1901]) have been described in the Ochlockonee River system, but it is not yet known if these other species are as genetically different from their congeneric relatives as is V. limi. On the other hand, Davis et al. (1981) reported low genetic distances among unionid populations in the southeastern United States including the Ochlockonee River drainage system. However, larvae of unionid bivalves are parasitic on fish; therefore extended dispersal ability is expected. No comparable vertebrate data are available since Avise and his colleagues did not collect samples from the Ochlockonee River system (Avise et al., 1987).

Morphology

The canonical discriminant and discriminant function analyses of shell morphology separated the genetically recognized Viviparus species with little overlap or misclassification. The continuous distribution of morphological data points scattered on the first and second canonical axes among the species, except between V. goodrichi and V. limi, suggests that some snails of one species resemble snails of another species. Therefore, the electrophoretic data were more definitive than shell morphological data in identifying the species. However, since overlap of the morphological measurements among the species was slight, shell morphology could be used to identify species or to compare with museum specimens when live snails were not available (see Chapter 3).

There are two reasons why morphological differences successfully characterized the species of Viviparus in this study. First, because the genotype of each individual was known, canonical discriminant analysis, which maximizes differences among groups, was used instead of principal component analysis, which only maximizes variance of orthogonal linear combinations of data. Second, curvilinear relationships were removed prior to the data analysis by logarithmic transformation. In fact, Viviparus georgianus possess allometric shell growth (Jokinen et al., 1982). Juvenile shells are not miniatures of adults; instead, juveniles change their shape as they grow. These allometric relationships were well expressed by linear combinations of $\log_e$-transformed data.

Intraspecific variation of shell morphology was size-related. In the principal component analysis, more than 98% of the variation was explained by the first principal component alone, which has all positive elements of the first eigenvector (a size component). Similarly, when only size-related variables were used, the first principal component accounted for a high proportion of the total morphological variation in other snails (Phillips et al., 1973) and crabs (Campbell and Mahon, 1974).

If environmental factors affect shell morphology, there are obvious implications for gastropod systematics (Vermeij, 1980). Several studies reported the effects of environmental factors on growth rate (Kemp and Bertness, 1984; Brown, 1985), shape (Spight, 1973; Kemp and Bertness, 1984), and color of snail shells (Neumann, 1959). Environmental differences could produce apparent morphological variation without a genetic basis. Such phenotypic plasticity in this study was due primary to shell weight and shell erosion. These morphological variations do not correspond with electrophoretic differentiation, suggesting that the variations were environmentally induced. Moreover, there is empirical evidence for environmentally induced shell weight and erosion differences. For example, Kemp and Bertness (1984) demonstrated that slowly growing snails developed thick, heavier shells. Ribi et al. (1986) showed that shell dissolution was a function of low calcium concentration of the water. However, available water chemistry data (Black and Brown, 1951) did not show clear differences between sites where the apex was eroded and sites where the apex was not eroded.

This work demonstrates marked genetic differentiation within and among drainage systems in freshwater species. The genetic discontinuity was not randomly distributed; hence, more pronounced differences were found among the three geographically separated Viviparus species. Moreover, significant intraspecific variation among the tributaries existed in V. goodrichi and V. georgianus. The large interspecific genetic subdivision in the Viviparus species appears to reflect biogeographic histories, rather than different types of environments. The intraspecific genetic differences can be explained by either geographical subdivision or selection; these forces are not easy to separate from each other.

CHAPTER 3

TAXONOMY AND SYSTEMATICS IN THE SOUTHEASTERN UNITED STATES

Introduction

The taxonomic status of Viviparus georgianus (Lea, 1837), a common freshwater snail in the eastern United States, has been questioned because of its variable shell morphology (Clench and Turner, 1956; Clench, 1962; Clench and Fuller, 1965; Thompson, 1984). Populations of V. georgianus are highly variable in morphology and color, although snails within a population are more similar to each other than to other populations. As a consequence, fourteen species and subspecies have been described based upon color and shell obesity or globoseness (see Appendix). In Chapter 2, genetic and morphological variation in the V. georgianus complex were studied in the southeastern United States. Eleven populations were clustered into three allopatric and genetically isolated groups (species) characterized by at least seven diagnostic loci among them out of the 38 allozyme loci studied. These groups were recognized as discrete species based on genetic, morphological, and ecological differences in Chapter 2. The phylogenetic species concept (Wiley, 1981) was applied to these allopatric groups, without direct assessment of reproductive compatibilities. The western species was distributed between the Choctawhachee River and the Apalachicola River systems. A second species was found only in the Ochlockonee River system. The eastern species was located in the Suwannee River, the Withlacoochee River in southern Florida, and the St. Johns River system.

Four species and subspecies, out of the fourteen described, are possible names for the three Viviparus species recognized in Chapter 2 (Fig. 3.1). V. georgianus was described by Lea (1837) from Hopeton, near Darien, Georgia in the Altamaha River system. Pilsbry (1916) described a new subspecies V. contectoides impolitus (= V. georgianus impolitus) from the Paint Rock River, Jackson Co., Alabama in the Tennessee River system. This subspecies has heavier and more shouldered shells than V. contectoides (= V. georgianus). In the same paper, he described V. contectoides compactus (= V. georgianus compactus), which is compactly coiled, from Dougherty [Co.?], Georgia in the Apalachicola River system. The latter subspecies name was subsequently corrected to V. contectoides limi because of homonyms (Pilsbry, 1918). Archer (1933) described a new subspecies V. contectoides goodrichi (= V. georgianus goodrichi), which has more globose shells than V. contectoides (= V. georgianus), from the Chipola River, near Marianna, Jackson Co., Florida in the Apalachicola River system.

The taxonomic value of shell characters is sometimes questionable because of convergence (Davis, 1982) and environmental effects (Vermeij, 1980; Crothers, 1982; see Chapter 2). The three species within the Viviparus georgianus complex were separated by multivariate techniques such as discriminant function and canonical discriminant analyses in Chapter 2. Multivariate morphometrics are often used when single morphometric and meristic characters are unreliable to discriminate taxa. Multivariate methods have been applied successfully to organisms in which species were difficult to

Fig. 3.1. Shell morphology variation of Viviparus spp. Top left to right: V. contectoides goodrichi, paratype, MCZ 92433; V. contectoides impolitus, holotype, ANSP 66701; V. contectoides limi, holotype, ANSP 327082; V. georgianus, holotype, USNM 106252. Bottom left to right: the western species from the Chipola River in Calhoun Co. (A2) and the Spring Creek (A3); the Ochlockonee species from Lake Talquin (O1); the eastern species from Lake Monroe (J3). Bar = 2 cm.

distinguish by classical taxonomic methods, such as mollusks (McDonald et al., 1991) and fish (Muoneke et al., 1991). The purpose of this study is to assign the three Viviparus species to their proper taxa. Also because these species can be separated by genetic and morphological criteria, the other purpose is to give the characteristics which can be used to discriminate these species.

Materials and Methods

Eleven collections comprising 355 snails were made from six drainage systems: Choctawhachee (collection abbreviation: C1), Apalachicola (A1, A2, and A3), Ochlockonee (O1), Suwannee (S1 and S2), Withlacoochee [in south Florida] (W1), and St. Johns (J1, J2, and J3) systems; details (localities, collection dates, sample sizes, and voucher numbers) are given in Chapter 2. Based on genetic data, the eleven collections were separated into three species (a western species: C1, A1, A2, and A3; an Ochlockonee River species: O1; and an eastern species: S1, S2, W1, J1, J2, and J3). In addition to these snails, 124 museum specimens from four regions of the southeastern United States (Table 3.1) were compared in morphology with the three Viviparus species.

The seven shell measurements [Fig. 2.3 in Chapter 2: shell height (SH), shell width (SW), two-whorl height (2WH), body whorl height (BWH), second whorl width (2WW), aperture height (AH), aperture width (AW)] were taken to the nearest 0.1 mm using vernier calipers. The seven variables were $\log_e$-transformed prior to analysis, to reduce curvilinear

Table 3.1. *Viviparus* museum specimens used for morphological comparison.

Locality	Species	Remark	No. snails examined	Voucher number[a]
Region 1 (Chipola River, Florida; 34 specimens)				
5 mi NE of Marianna Jackson Co.	*V. contectoides goodrichi*	paratypes	4	MCZ 92433
Calhoun Co.	*V. georgianus*		10	MCZ 191879
2 mi E of Clarksville [Calhoun Co.]	*V. georgianus*		20	MCZ 191880
Region 2 (southwestern Georgia; 50 specimens)				
Dougherty [Co.]	*V. contectoides limi*	holotype	1	ANSP 327082
Dougherty [Co.]	*V. contectoides limi*	paratypes	2	ANSP 327081
6 mi W of Alberny [Dougherty Co.]	*V. contectoides*		27	ANSP 243568
15 mi SE of Dawson Terrell Co.	*V. georgianus*		10	MCZ 75004
3 mi S of Leary Calhoun Co.	*V. georgianus*		10	MCZ 109628
Region 3 (Altamaha River, Georgia; 41 specimens)				
Darien [McIntosh Co.]	*V. georgianus*	holotype	1	USNM 106252
Darien	*V. georgianus*	paratypes	3	MCZ 186792
Darien	*V. georgianus*		2	USNM 27763
Darien	*V. georgianus*		8	USNM 106246[b]
Darien	*V. georgianus*		4	USNM 106254[b]
Lower Altamaha R.	*V. georgianus*		8	MCZ 237898
Altamaha R.	*V. georgianus*		5	USNM 27743[b]

Table 3.1. (continued)

Locality	Species	Remark	No. snails examined	Voucher number
Region 4 (Jackson Co., Alabama; 9 specimens)				
Paint Rock River	*V. contectoides impolitus*	holotype	1	ANSP 66701
Paint Rock River	*V. contectoides impolitus*		8	ANSP 327079

[a]Museum of Comparative Zoology of Harvard University, Cambridge (MCZ); Academy of Natural Sciences of Philadelphia, Philadelphia (ANSP); National Museum of Natural History, Washington (USNM).
[b]Lots include several small shells (< 13.0 mm in shell height) which were not examined.

relationships among variables. Two multivariate methods were used to identify species: discriminant function analysis and canonical discriminant analysis (also called canonical vectors or canonical variates analysis). Both analyses, which are similar in principle (James and McCulloch, 1990), were used to find linear functions (or combinations) of morphological variables with coefficients that maximize statistical distances among the three Viviparus species. The variable defined by the first linear combination is the first canonical variable in canonical discriminant analysis. The second canonical variable is obtained by finding the second linear combination uncorrelated with the first canonical variable. The two analyses are different in assumptions and data presentation. Discriminant function analysis can use either the individual within-group covariance matrices or the pooled covariance matrix to make a classification criterion. The criterion can be applied to test data (museum specimens for this study) and probabilities of group membership are calculated for each observation. The probability means the likelihood that the identification of a given specimen is correct. Canonical discriminant analysis assumes a common covariance matrix for classes (species in this study). Canonical variable scores of each subject can be plotted graphically, unlike discriminant function analysis. The canonical coefficients can be applied to a second data set to calculate their canonical variables. Both analyses were employed to obtain classification results and graphical presentation in this study. DISCRIM and CANDISC procedures of SAS were used for multivariate analyses (SAS

Institute Inc., 1990). The canonical variable for each museum specimen was calculated as the sum of each shell morphological variable (= $\log_e$-transformed value minus its total-sample mean) multiplied by its raw canonical coefficient (= total-sample standardized canonical coefficient divided by total-sample standard deviation) using the results of the canonical discriminant analysis of the 355 snails (Table 3.2).

A likelihood ratio test (Anderson, 1984, Chapter 10) on the within-group covariance matrices showed significant departure from homogeneity among groups. Therefore, the within-group covariance matrices were used for the discriminant function analysis. If the lack of homogeneity affects group configuration significantly, then the canonical discriminant analysis is not reliable. However, the discriminant function analysis (which is similar in principle to canonical discriminant analysis [James and McCulloch, 1990]), gave similar misclassification proportions when based on either a pooled covariance matrix (13.2% misclassification) or on separate within-group covariance matrices (9.6%). This suggests that pooling of covariance matrices did not significantly affect analyses and results of the canonical discriminant analysis are valid.

Results

Although ranges of morphological variables overlapped considerably among the three Viviparus species (Table 3.2), more than 90% of the original 355 Viviparus snails were correctly classified by discriminant function analysis using the within-group covariance matrices (Table 3.3). Most of the

Table 3.2. Canonical discriminant analysis, means, and standard deviations of seven morphological variables for three Viviparus species. Abbreviations: SH, Shell height; SW, shell width; 2WH, two-whorl height; BWH, body whorl height; 2WW, second whorl width; AH, aperture height; AW, aperture width.

Variable	Natural logarithmic mean(standard deviation)				Total-sample standardized canonical coefficient		Total canonical structure	
	Total	Western	Ochlockonee R.	Eastern	CAN1	CAN2	CAN1	CAN2
SH	3.191(0.261)	3.204(0.286)	3.176(0.284)	3.188(0.236)	0.67	2.07	0.047	0.010
SW	3.021(0.231)	3.074(0.254)	2.927(0.243)	3.018(0.198)	3.81	-3.87	0.261	-0.042
2WH	3.130(0.253)	3.152(0.282)	3.100(0.272)	3.124(0.223)	-7.98	-0.99	0.088	0.011
BWH	3.025(0.246)	3.060(0.279)	2.977(0.261)	3.017(0.212)	-3.05	0.88	0.143	0.012
2WW	2.752(0.247)	2.787(0.276)	2.669(0.271)	2.756(0.210)	2.69	-1.64	0.188	-0.074
AH	2.728(0.216)	2.810(0.235)	2.658(0.224)	2.695(0.181)	3.28	6.29	0.318	0.151
AW	2.517(0.217)	2.579(0.236)	2.417(0.236)	2.510(0.180)	0.99	-2.65	0.310	-0.024

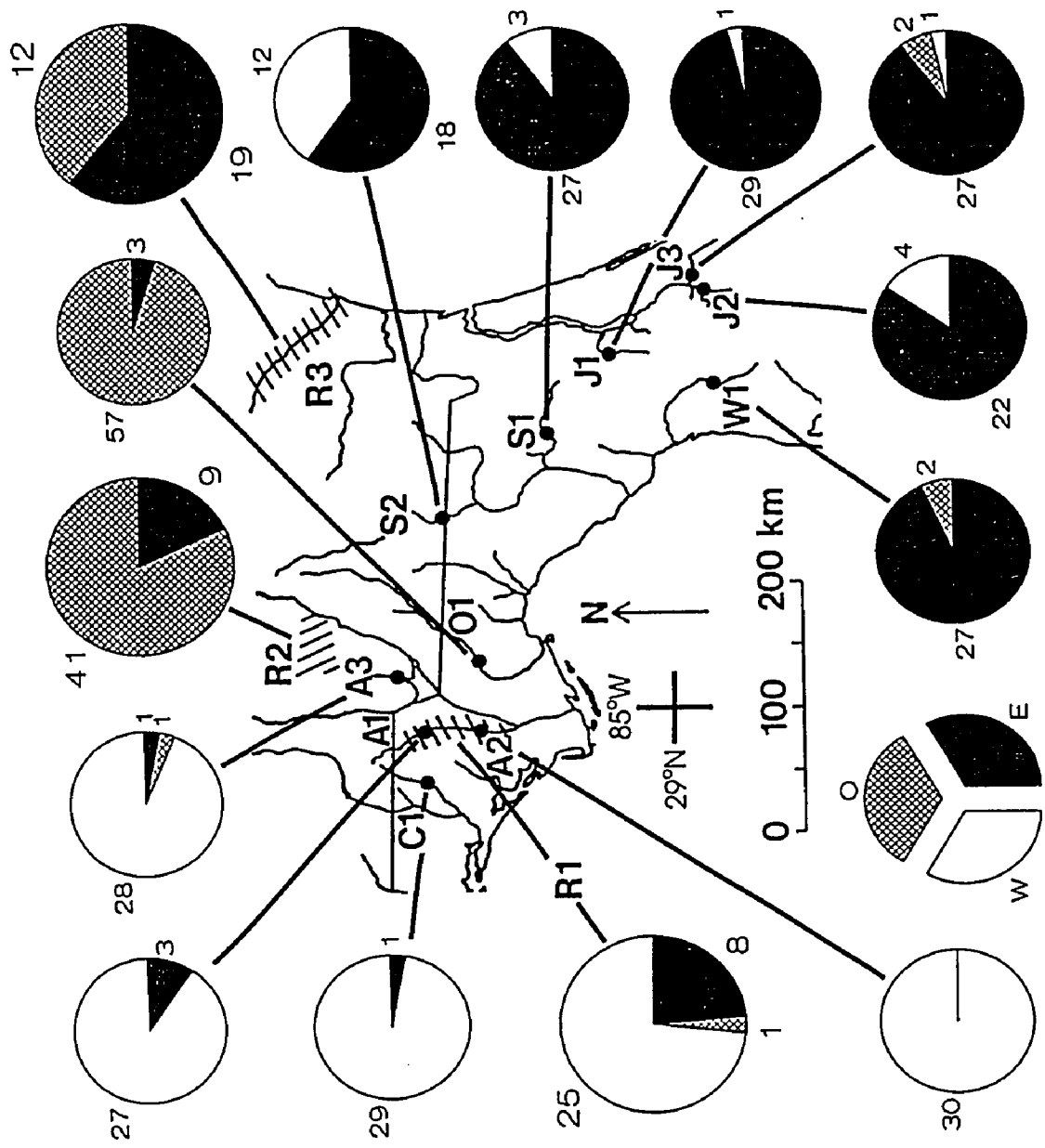
Table 3.3. Discriminant function analysis for three Viviparus species based on seven morphological variables.

From species	Number of individuals (%) classified into species			
	Western	Ochlockonee R.	Eastern	Total
Western	114 (95.0)	1 (0.8)	5 (4.2)	120 (100.0)
Ochlockonee R.	0 (0.0)	57 (95.0)	3 (5.0)	60 (100.0)
Eastern	21 (12.0)	4 (2.3)	150 (85.7)	175 (100.0)
Total	135	62	158	355

misclassification was due to the eastern snails wrongly sorted into the western species at site S2 (Fig. 3.2). This indicates that the classification criterion based on the seven morphological characters is high enough to identify species in the 355 snails and the 124 museum specimens by discriminant function analysis. The three Viviparus species were also separated graphically by canonical discriminant analysis of the seven variables with little overlap between the eastern and western species (Fig. 3.3, A). Total-sample correlations between the canonical variables and the original morphological variables were listed in Table 3.2. Although the first canonical variable had high positive correlations with aperture height, aperture width, shell width, and second whorl width, and separated the Ochlockonee and western species (Table 3.2), the first canonical variable scores of the eastern species overlapped between the other two species (Fig. 3.3, A). The first canonical variable indicated that the western species had a more globose shell with larger aperture than the Ochlockonee species. The eastern species was discriminated by the second canonical variable, which had a high positive correlation with aperture height (Table 3.2). This means that the western and Ochlockonee River species have larger aperture height than the eastern species.

The discriminant function and canonical discriminant analyses can be used for morphological comparison with museum specimens. Therefore, both multivariate analyses were applied to museum samples from four regions. In discriminant function analysis, individual shells were classified into the three

Fig. 3.2. Classification of snails from 11 sites (small circles) and of museum specimens (large circles) from regions 1 through 3 (R1-3: hatched) in the southeastern United States into the three Viviparus species (W, western; O, Ochlockonee River; E, eastern) by discriminant function analysis of seven shell morphological variables. Numbers beside the pies are sample size. The western species: Homes River (C1), Chipola River in Jackson Co. (A1), Chipola River in Calhoun Co. (A2), and Spring Creek (A3); the Ochlockonee River species: Lake Talquin (O1); the eastern species: Santa Fe River (S1), Withlacoochee River of Suwannee (S2), Withlacoochee River in southern Florida (W1), Silver River (J1), Wekiva River (J2), and Lake Monroe (J3).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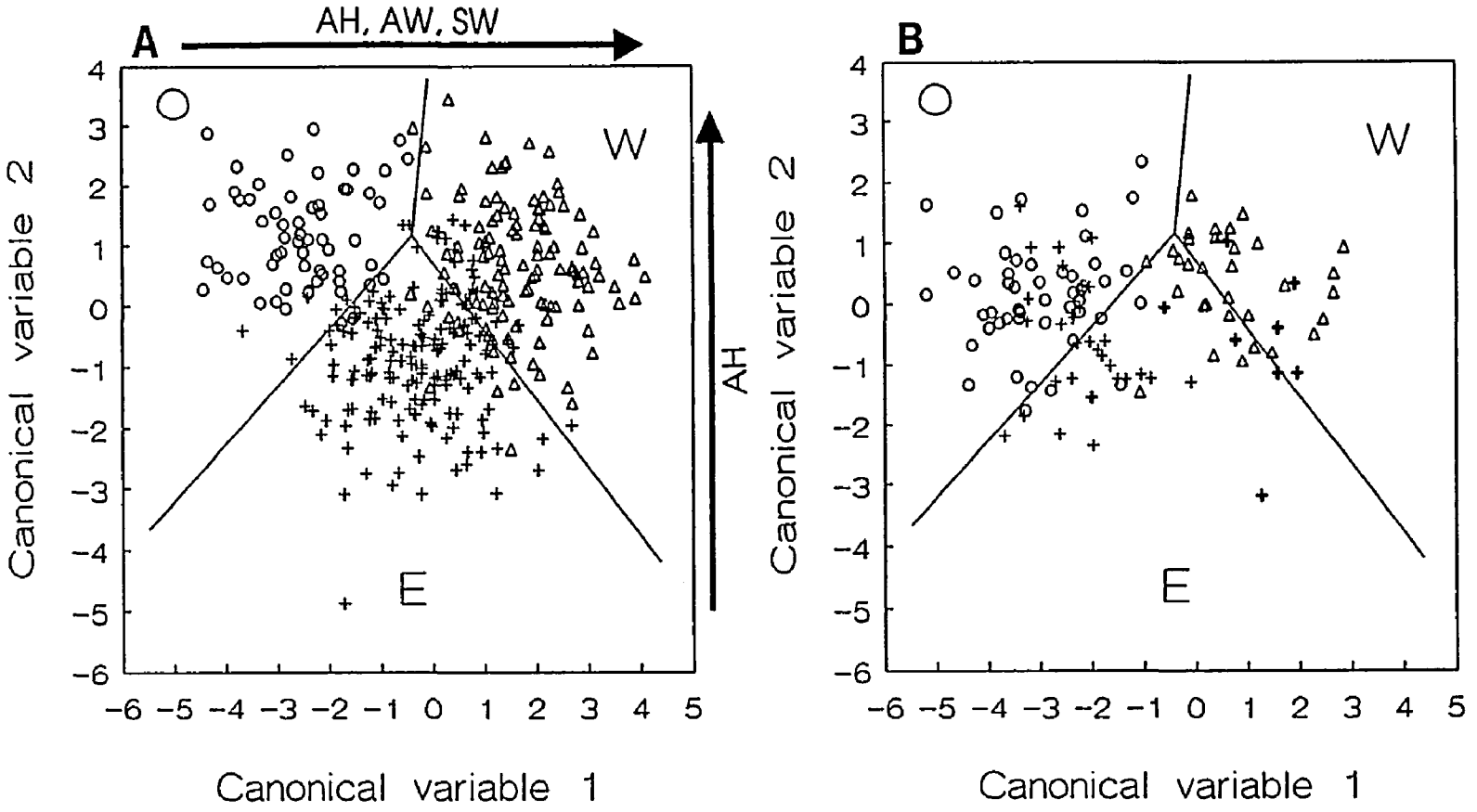

Fig. 3.3. (A) Separation of the three Viviparus species (▵: western [W]; o: Ochlockonee River [O]; +: eastern [E]) by the first and second canonical variables of seven shell morphological measurements. To compare with museum specimens, perpendicular bisectors of lines connecting the centroids of the three species clusters are drawn. (B) Canonical scores for museum specimens from the four regions (▵, Region 1 [Chipola River]; o, Region 2 [southwestern Georgia]; +, Region 3 [Altamaha River]; +, Region 4 [northeastern Alabama]), calculated from the canonical discriminant functions of (A). The lines of (A) are repeated.

species using the classification criterion from the 355 snails (Fig. 3.2). Most snails (74%) including all paratypes (the holotype was not used) from Region 1 (the Chipola River), which is the type locality for Viviparus contectoides goodrichi, were sorted into the western species from the same river in the Apalachicola River drainage system as expected (Fig. 3.2). Their first and second canonical variable scores fell into the range of the western species (Fig. 3.3, B). Surprisingly, most snails (82%) including the holotype and both paratypes from Region 2 (southwestern Georgia) in the Apalachicola River system, which includes the type locality of V. contectoides limi, were classified not into the western species from the same drainage system but into the Ochlockonee River species from the different drainage system (Fig. 3.2). Most canonical variable scores were in the range of the Ochlockonee River species yet some were in the range of the eastern species or out of ranges of the all three species (Fig. 3.3, B). A majority (61%) of the snails from Region 3, which is the type locality for Viviparus georgianus, were sorted into the eastern species. However, the holotype and two out of three paratypes of Viviparus georgianus were classified into the Ochlockonee River species. In fact, the distribution of canonical variable scores of the snails from Region 3 overlapped the eastern and Ochlockonee River species (Fig. 3.3, B). Classification of snails from Region 4 (northeastern Alabama) was split among the three species: four snails into the western species; two into the Ochlockonee River species; three including the holotype of V. contectoides impolitus into the eastern species. Their canonical variable

scores were scattered but not near the middle of the Ochlockonee River species range and the center of the distribution of the snails from Region 4 was in the area of overlap between the eastern and western species.

The great morphological similarity and geographical proximity found between the Ochlockonee River species and Viviparus contectoides limi suggests that they are the same species (Viviparus limi Pilsbry). The known distribution of this species is in the Ochlockonee River and at the eastern end of the Chattahoochee River in the Apalachicola drainage system. There were no other synonyms for V. limi based on morphological comparison in this study and the localities of the other 13 Viviparus (sub)species.

Distribution alone suggests that the western species and Viviparus contectoides goodrichi are the same, and multivariate morphometrics confirmed this. Although some of specimens from Region 4, where V. contectoides impolitus was described earlier than V. contectoides goodrichi, were classified into the western species, the split classification of snails from Region 4 did not provide enough evidence to synonymize them. Therefore, Viviparus goodrichi Archer should be used for the western species. Current known distribution is in the Apalachicola and Choctawhachee river systems.

Since most of the snails from the Altamaha River were classified into the eastern species and the river is close to eastern Florida, the eastern species should be called Viviparus georgianus (Lea). However, intermediate canonical variable scores for snails from the Altamaha River may suggest the

existence of a fourth allopatric species, or a genetically different population of V. georgianus, or several sympatric species in that river. V. georgianus, the eastern species of the present study, is distributed in the Altamaha River and eastern and southern Florida.

Discussion

Although the ranges of the seven morphological variables used in this study overlapped considerably among the western, Ochlockonee River, and eastern species, more than 90% of the snails were correctly classified by discriminant function analysis of only seven morphological measurements. Moreover, canonical discriminant analysis separated the three species with little overlap. Success in classification and separation was not only due to existence of morphological differences among species, but also due to the two multivariate analyses which maximize among-group discrimination because group membership of the snails was known based on electrophoretic results prior to the analysis, except for the museum specimens. The results indicate that discriminant function and canonical discriminant analyses are very powerful in systematic studies. Using these techniques, McDonald et al. (1991) discriminated three species of Mytilus morphologically by canonical discriminant analysis of electrophoretically identified mussels. The degree of overlap of canonical variable scores for the morphological analysis of the 355 snails indicates that shell morphology alone is less informative than genetic data, which showed complete separation of the three Viviparus species (Chapter 2).

Some difficulties associated with comparison of the original 355 and museum specimens are that new specimens always bring some additional variation due to temporal and geographical differences or misidentified individuals. Sometimes, identification of museum specimens by discriminant function analysis could be wrong. Percentages of misclassification of museum specimens would be similar to or larger than 10% of my original data. Discriminant function and canonical discriminant analyses are designed to work best for the original data, not for test data. For example, if the test data includes different groups which are not in the original data set to make a discrimination criterion, subjects belonging to new groups will be forcibly classified into the original groups. Therefore, results of multivariate analyses applied to test data have to be interpreted carefully because results of new groups are unpredictable.

Beyond these difficulties, morphological similarity seen between snails from the Ochlockonee River and Region 2 indicates that they are conspecific Viviparus limi. More genetic studies are need to clarify the questions posed by this study such as the status of Viviparus contectoides impolitus and genetic relationships between the Altamaha River and eastern Florida populations. Moreover, northeastern U.S. populations of V. georgianus need to be studied for complete revision of the V. georgianus complex.

Systematics and Distribution

Since electrophoretic comparison among populations in eastern Georgia (the type locality of Viviparus georgianus [Lea]), eastern Florida, and the northeastern United States is necessary to review Viviparus georgianus, diagnosis and distribution of V georgianus are not listed.

Viviparus goodrichi Archer, 1933

Viviparus contectoides goodrichi Archer, 1933 (Archer, 1933, p. 18-20, Figs. 1-3).

Diagnosis. Archer (1933) described the morphology of Viviparus goodrichi compared to V. contectoides Binney (= V. georgianus) and V. limi: V. goodrichi is more globose with more broadly shouldered whorls than V. contectoides; V. limi is more elongate with more compactly coiled whorls than V. goodrichi. Canonical discriminant analysis in this study revealed the following differences: V. goodrichi has a more globose shell with larger aperture than V. limi; V. goodrichi and V. limi have larger aperture length than V. georgianus in eastern Florida. These morphological differences cannot distinguish V. goodrichi, V. limi, and V. georgianus all the time. However, the three Viviparus species can be distinguished reliably using allozyme characters in Chapter 2. These characters are migration distances relative to known allozymes, and thus identifying an unknown sample of Viviparus snails will require comparison with snails from the reference sites whose genetic structures are

known. Relative to the common allozymes in V. limi, in V. goodrichi the common βGALA, GAPDH, βGLUR, PEP-GL, PK, TAT, and TPI allozymes migrate faster, while the common GPI, IDDH, PGK, and XDH allozymes migrate slower. Relative to the common allozymes in V. georgianus, in V. goodrichi the common ALAT-1, βGLUR, PEP-GL, and PK allozymes migrate faster, while the common βGALA, GPI, and IDDH allozymes migrate slower. Relative to the common allozymes in V. georgianus, in V. limi the common ALAT-1, βGAL, βGLUR, GPI, PEP-GL, IDDH, PGK, and XDH allozymes migrate faster, while the common βGALA, GAPDH, HK, PNP, TAT, and TPI allozymes migrate slower (Table 2.4).

Type locality. Spring fed stream tributary to the Chipola River, 5 miles northeast of Marianna, Jackson Co., Florida, USA.

Type specimen. Holotype: Museum of Comparative Zoology (MCZ) No. 92432. Paratypes: MCZ 92433.

Distribution. The Apalachicola and Choctawhachee river systems. No other Viviparus species was found in the Chipola and Homes rivers in this study.

Viviparus limi Pilsbry, 1918

Viviparus contectoides compactus Pilsbry, 1916 (Pilsbry, 1916, p. 42). non compactus Kobelt 1906.

Viviparus contectoides limi Pilsbry, 1918 (Pilsbry, 1918, p. 71).

Diagnosis. Morphological characters cannot distinguish V. goodrichi, V. limi, and V. georgianus all the time, as described for V. goodrichi. However, the three Viviparus species can be distinguished reliably using allozyme characters as described for V. goodrichi.

Type locality. Dougherty [Co.?], Georgia, USA.

Type specimen. Holotype: Academy of Natural Sciences in Philadelphia (ANSP) No. 327082. Paratypes: ANSP 327081.

Distribution. The Ochlockonee River and eastern end of the Chattahoochee River in the Apalachicola drainage system. No other Viviparus species was found in Lake Talquin, Florida in this study.

CHAPTER 4

SUMMARY AND CONCLUSIONS

Genetic and morphological variation in a morphologically highly variable freshwater snail [Viviparus georgianus (Lea)] were studied in the southeastern United States. Eleven populations were clustered into three genetically isolated allopatric species characterized by at least 7 diagnostic loci out of the 38 loci studied. Gene flow between drainage systems seemed to be low, and considerable intraspecific differentiation among sites was also observed. These results corroborate earlier studies indicating that freshwater snails are subdivided genetically within and among drainage systems. Based on Wright's (1951) F-statistics, freshwater Viviparus prosobranchs were more differentiated than marine counterparts which lack free swimming larvae. This may indicate that some unique biological (adaptive) or abiological (physical) mechanisms that prevent gene flow exist in freshwater. Canonical discriminant analysis of nine morphological measurements separated the three species (which were recognized by electrophoresis) with little overlap. This result has important implications in molluscan systematics. Combination of genetic and morphological data from each snail species made possible a comparison of my samples with museum specimens (only shells) by proper multivariate analyses. There are many systematic problems which cannot be solved simply by comparing museum dry shell collections. Acquisition of both genetic and morphological information may answer many taxonomic contradictions. Based on discriminant function and canonical

discriminant analyses of seven shell morphological variables with museum specimens, the three Viviparus species appear to be Viviparus goodrichi Archer, Viviparus limi Pilsbry, and Viviparus georgianus (Lea). The three species can be distinguished by the following morphological characteristics: V. goodrichi has a more globose shell with larger aperture than V. limi; V. georgianus has a shorter aperture height than the other species. Also, these species can be identified reliably using allozyme characters. V. goodrichi is distributed in the Apalachicola and Choctawhachee river systems. V. limi is found in the Ochlockonee River and at the eastern end of the Chattahoochee River in the Apalachicola River system. V. georgianus is distributed in eastern Florida and Georgia. For complete revision of the V. georgianus complex, populations from the entire distribution need to be examined.

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APPENDIX

FOURTEEN SPECIES AND SUBSPECIES OF THE VIVIPARUS GEORGIANUS COMPLEX SYNONYMIZED BY CLENCH AND FULLER (1965)

Paludina georgiana Lea 1837 (Hopeton, near Darien, [McIntosh Co.], Georgia), [holotype, USNM 106252; paratype, MCZ 186792].

Paludina linearis Kuster 1852 (Simpson Creek Lake, East Florida).

Paludina wareana Kuster 1852 ([Lake Weir, Marion Co., Florida]).

Vivipara haldemanniana Frauenfeld 1862 (Black Creek [Clay Co.], East Florida).

Vivipara inornata Binney 1865 (near Chopatilo, Mexico), [lectotype, MCZ 234704; paratype, MCZ 20512].

Vivipara contectoides Binney 1965 (Florida), [lectotype, MCZ 74393; paratypes, USNM 27756].

Vivipara georgiana fasciata Tryon 1870 ([Florida]).

Paludina inornata Binney [in] Fischer and Crosse 1890 [lectotype, MCZ 234704; paratypes, USNM 9168].

Vivipara georgiana altior Pilsbry 1892 (aboriginal shell heap, left bank of Hitchen's Creek, near entrance of St. Johns River into Lake George [Putnam Co.], Florida), [lectotype, ANSP 63420a].

Viviparus georgiana limnothauma Pilsbry 1895 (aboriginal shell heap, left bank of Hitchen's Creek [near entrance of St. Johns River into Lake George, Putnam Co.], Florida and in 2 fathoms, Lake George [Florida]), [lectotype, ANSP 70052a].

Viviparus walkeri Pilsbry and Johnson 1912 (Juniper Creek, Lake Co., Florida), [lectotype, ANSP 70053a].

Viviparus contectoides impolitus Pilsbry 1916 (in marsh, Paint Rock iver, Jackson Co., Alabama), [holotype, ANSP 66701a].

Viviparus contectoides compactus Pilsbry 1916 (Dougherty [Co.?], Georgia), [holotype, ANSP 27731], non compactus Kobelt 1906.

Viviparus contectoides limi Pilsbry 1918 (Dougherty [Co.?], Georgia), [holotype, ANSP 27731], [new name for V. compactus Pilsbry, non Kobelt].

Viviparus georgianus goodrichi Archer 1933 (spring-fed stream triburary of the Chipola River, 5 mi NE of Marianna, Jackson Co., Florida], [holotype, MCZ 92432].

VITA

Masaya Katoh was born in Tokyo, Japan on February 26th, 1959 and had lived in Tokyo for about 20 years. He graduated from Tachikawa High School in Tokyo in 1977. In 1980, he moved to Okinawa to study tropical marine biology. He received his Bachelor of Science and Master of Science degrees in Marine Sciences from the University of the Ryukyus, Okinawa in 1984 and 1986, respectively. He entered the Ph.D. program in the Department of Zoology and Physiology at L.S.U. in 1986. His research publications include a study of life history in the cowry Cypraea annulus (Marine Biology 101: 227-234) based on his master's thesis. The other with Dr. D. W. Foltz concerns biochemical evidence for the existence of a null allele at the leucine aminopeptidase-2 (*Lap-2*) locus in the oyster Crassostrea virginica (Genome 32: 687-690). Another two papers have been accepted recently. One with Mr. S. K. Sarver and Dr. D. W. Foltz studies apparent overdominance of enzyme specific activity in two marine bivalves (Genetica, in press). The other with Dr. W. B. Stickle, Dr. D. W. Foltz, and Ms. H. L. Nguyen concerns genetic structure and mode of reproduction in five species of sea stars from the Alaska coast (Canadian Journal of Zoology, in press). He is planning to submit two manuscripts for publication based on this dissertation. Currently, he is a candidate for the Degree of Doctor of Philosophy in Zoology.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Masaya Katoh

Major Field: Zoology

Title of Dissertation: Genetic and Systematic Study of Viviparus georgianus (Lea), a Freshwater Snail Species Complex

Approved:

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